SOME PARASITES OF SIMULIUM LARVÆ AND THEIR EFFECTS ON THE DEVELOPMENT OF THE HOST.[1]

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During the spring of the present year (1911) while studying entomology at the Bussey Institution of Harvard University, I made numerous collections of *Simulium* larvæ, which are extremely abundant in the neighboring streams, with the intention of studying the development of the imaginal discs which are unusually well defined in this genus of diptera. As, however, I found that a large percentage of these interesting larvæ were heavily parasitized by two very different organisms, namely a worm and a protozoön, I turned my attention rather more directly to these and their effects on their hosts, during the few weeks intervening between my first discovery of the parasites and the pupation of the insects.

Before giving any details I wish to take this opportunity to offer my sincere thanks to Professor Wheeler, who by his kind suggestions and advise enabled me to bring together the following facts, which, though very incomplete in form, do not appear to have been recorded before. My thanks are also due to Professor Johannsen for naming the species of *Simulium* larvæ and to Professor T. H. Montgomery who identified the worm parasite as a species of *Mermis*.

Life History and Structure of Simulium Larvæ.

A brief summary of the structure, and mode of life, of the *Simulium* larvæ may not be out of place here.

If during the months of March to May one examines the rocks or vegetation in any swiftly flowing stream in the neighborhood of Forest Hills, Mass., especially where its bed causes a small cascade, one will, in all probability, observe a large, dark, gelatinous mass where the current is swiftest and the water

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shallowest. On closer examination the mass will be seen to consist of numerous curiously shaped larvæ standing upright on the rock, to which the hind end of the abdomen is firmly attached. These larvæ, the largest known North American species of which measure when mature some 12 mm., have the following characters: The soft-skinned body is more or less cylindrical, though the posterior third is somewhat swollen. The segments are poorly defined but the first three behind the head, namely, the thoracic segments, are usually distinctly marked off from the following abdominal segments.

The prothoracic segment bears a single leg (Plate I., Fig. 1) which is apparently two-jointed; the distal joint is small and retractile and terminates in a sucker which is armed with numerous hooks. No other segment bears any trace of legs, with the exception of the apical abdominal segment where the pair of anal prolegs of some other larvæ is represented by a powerful, armed disk-like sucker, by means of which the larva firmly attaches itself to rocks or vegetation when at rest. The skin is usually of a greenish gray color in immature larvæ, but gives place to a reddish brown as they mature.

The head is sub-cylindrical and is usually of a darker color than the rest of the body. Its chitinous integument is much denser and less elastic than that covering the remainder of the body. This results, during growth, in an ecdysis of the head capsule alone, being necessary more frequently than that of the general cuticular covering of the body. The new head capsule exposed after such an ecdysis is perfectly pigmentless in *Simulium hirtipes*, though dark spots soon form on its vertex (Plate I., Fig. 2, *a*, *b* and *c*), followed by a gradual infuscation of the whole surface. Individuals were observed in which the almost black head capsule was half removed, exposing the new pigmentless capsule, entirely independently of the body cuticle, the ecdysis of which was never observed, except at pupation.

On either side of the head are two black eye spots and on the anterior border are two lateral fan-like organs borne on elongated pedicels, which are used by the larva in procuring food. These fans consist of numerous curved rakes (Plate I., Fig. 3) bearing on one side long stiff cilia which, when the fan is expanded, stretch from one rake to the next, thus forming a very fine strainer which allows water to pass through it, but retains any small organisms, such as the diatoms, on which the larva subsists. By means of a curious flicking motion these fans can be closed and their contents brushed into the mouth orifice. Here are situated two large glands (Plate IV., Figs. 9 and 10 (*d*)) on the dorsal side of the pharynx, whose function appears never to have been determined. They are covered with an apparently porous membrane which is clothed with short stout hairs. It would seem that they secrete some sticky material onto these hairs which removes the particles of food from the rakes when the latter are brought in contact with them.

It is usually stated that the fans are used to set up currents in the water and thus sweep food toward the mouth. My observations, however, lead me to believe that they act as a "strainer" and this is supported by the fact that, living as these larvæ do, in the swiftest currents, such movements would be useless.

The salivary glands are very large and secrete powerful silken threads which are used by the larvæ as anchor lines to hold them in an upright position no matter how strongly the current of water may flow.

Although the larvæ appear to be very sluggish, they can move about actively on the rocks by a looping motion similar to that of the geometrid larva. It is interesting to note the care with which these larvæ, when thus moving about, assure themselves that the adhesive disc of the prothoracic leg is firmly attached before relaxing the anal disc and *vice versâ*. Respiration is accomplished by means of retractile finger-like blood gills, normally three in number, which are situated on the dorsal surface of the last abdominal segment just above the anus. These gills, which can be distended at will by the insect, by means of blood pressure, are apparently inadequate for use in any but rapidly flowing water which contains plenty of oxygen, for if the larvæ be placed in a jar of still water very few will survive for more than eight hours, though life may be prolonged for two or three days by placing them in small numbers in Petri dishes containing only sufficient water to just cover them.

The form and size of the imaginal discs, or histoblasts, will here be described in detail as they are of especial interest in connection with the parasites.

The thoracic histoblasts are twelve in number and are very large and conspicuous in normal larvæ as they approach full growth (Plate I., Fig. 4).

The six discs to be found on each side of the thorax are the following:

1. Adult prothoracic leg histoblast—situated at the base of the larval prothoracic leg.

2. Adult mesothoracic leg histoblast—situated ventro-laterally on the mesothoracic segment.

3. Adult metathoracic leg histoblast—situated ventro-laterally on the metathoracic segment.

4. Adult wing histoblast—situated dorso-laterally on the mesothoracic segment This in the mature larva is by far the largest disc and it soon comes in contact with the mesothoracic leg disc.

5. Adult halteric histoblast This in the early stages is almost as large as the wing disc, but its growth and development are very slow and it soon disappears under the rapidly expanding wing disc.

6. Pupal respiratory tuft histoblast. This is situated on the prothorax and in its young stages has the appearance of being quite homologous with a prothoracic wing. It, however, soon begins to take on a definite form, and the comparatively stout tracheal tubes can be seen growing and lengthening beneath the transparent cuticle till they become coiled up as indicated in Plate I., Fig. 4.

It should be noted that this histoblast is not in the true sense of the word an "imaginal" disc since the respiratory filaments are exclusively pupal organs, and have no equivalent structure in any adult. Their similarity to a prothoracic wing destitute of the wing membrane is of interest. A few days before pupation this "pupal" histoblast begins to darken. Pigmentation commences at the apex of the folded tubes and slowly works backwards toward the base when the disc takes on the appearance shown in Plate III., Fig. 8*b*. Later, immediately prior to pupation, the cuticle over this disc ruptures and liberates the fully formed and now functioning filaments.

The growth of these various histoblasts causes the thorax to swell considerably, thus giving the body the appearance of being constricted in the middle.

Internally there is comparatively little tissue. The abdomen contains the alimentary tract, and the much elongated salivary glands which lie normally in a ventro-lateral position with regard to the alimentary tract. The sexual organs are small and not easily found even in serial sections. The remaining portions of the body cavity are filled with blood plasma in which is suspended a quantity of fat body, mainly collected near the apex of the abdomen and causing this region to become slightly swollen. As the larvæ mature this fatty tissue very materially increases, and when dissected out, is of a stringy nature.

Methods.

Most of the larvæ were killed as soon as they were brought into the laboratory, but a few of the more heavily parasitized ones were kept alive in running water by covering the mouth of a jar with fine netting and introducing a piece of rubber pipe into the jar, through which tap water was run. In this manner specimens were kept alive for several days.

The following killing fluids were found to be the most satisfactory among several tried:

1. *Hot Water.*—Water was just brought to the boil when the larvæ were immersed and allowed to cool in the water. This method was most unsatisfactory from a histological point of view, but it had the advantage of leaving the skin as transparent as in its normal condition. It was also possible to dissect larvæ thus killed.

2. *Gilson's Fluid.*—This was used hot as described above and gave good results, but had the disadvantage of making staining with the hæmatoxylins difficult.

3. *Kahle's Fluid*, consisting of 30 parts water, 15 parts 96 per cent. alcohol, 6 parts formalin (40 per cent.), 1 part glacial acetic acid. This fluid has been recommended by W. Kahle (1908) and proved to be superior to Gilson's fluid both for

fixing and staining, and in the end was exclusively used. It was also used hot.

The chief advantage of both of these fluids was that, besides giving excellent histological preparation, they caused the histoblasts to turn milky white so that their position and form could be readily observed immediately after the specimens were killed.

Staining.—Heidenhain's iron hæmatoxylin combined with orange G was almost entirely used as it gave the best differentiation, though staining was rather slow. Replacing orange G with eosin accelerated staining but differentiation was less precise.

Mermis sp. Parasitizing Simulium Larvæ.

A number of larvæ were placed in a jar of water and left over night. The following morning I was surprised to see several white worms moving about at the bottom of the jar. It was evident that these had come out of the *Simulium* larvæ, so I went out to a ripple where the larvæ were particularly abundant and examined the colonies on several stones. I then noticed the large size of many individuals, and on examining these I found that many of them had a worm coiled up within the abdomen (Plate I., Fig. 5). When a quantity of material was brought into the laboratory it was found that these parasitized larvæ were much more sluggish than their healthy companions, which rapidly explored the jar in which they were confined, with their peculiar looping gait. The parasitized individuals, however, seemed much less concerned as to their new surroundings, and many of them were soon motionless, standing upright on the bottom and sides of the jar with their rakes expanded, ready to catch any food which might float their way. Here is a possible explanation of their larger size. Owing to their curious form of feeding it is evident that the more sluggish an individual is the more food it can obtain, and should it grow but a little larger than its companions, it will reach above their innumerable outstretched rakes, so that its food supply will be very materially increased.

It must not however be taken for granted that all parasitized larvæ were larger than their healthy companions, for some remained quite small, whereas many of the larger larvæ showed no signs of worm parasites. As a general rule, however, the largest larvæ were found to contain one or more worms coiled up within the abdomen. One would naturally expect this rule not to be very constant, if, as conjectured, it is simply due to a more sluggish temperament and increased appetite on the part of the parasitized larvæ.

A case of a *Mermis* parasitizing ants was described by Professor Wheeler ('07) and here also he noticed a great increase in size of the host due to a greatly increased appetite during the larval stage.

A number of the worms were dissected out and sent to Professor T. H. Montgomery who pronounced them to belong to an undetermined species of *Mermis*.

Retardation of Development of the Histoblasts Due to Mermis.

On making a closer examination of the parasitized larvæ a far more remarkable effect than that of increased size was noticed, for it was found that the presence of *Mermis* parasites, no matter in what numbers, has a direct effect on the development of the histoblasts. In a normal larva of about 10–10.5 mm. which is the maximum length, and is attained immediately prior to the blackening of the respiratory filament histoblasts, the latter are quite large and owing to their white color readily visible to the naked eye; especially when the larva has been killed in Kahle's or Gilson's fluid (Plate II., Fig. 6). If a parasitized larva of the same, or greater, size be examined no trace of the histoblasts can be discovered with the naked eye, and can only be detected with difficulty under a dissecting lens. Under the low power of a compound microscope, however, they are seen to be represented by small white traces of the organs which should at this time be far advanced in development (Plate II., Fig. 7). A close examination fails to reveal any differentiation of these retarded histoblasts into the component parts of the adult, or pupal, organ.

The parasitized larva rarely develops beyond this stage, though I have observed specimens which were turning reddish brown, and contracting slightly as a healthy larva would, shortly before maturation. In some cases, even, the rudimentary respiratory filament histoblast begins to darken in color.

I examined numerous specimens from the same stream at Forest Hills, and, with slight variations in intensity, they all showed this nearly complete suppression of the imaginal discs. Some weeks later I chanced to pass a small stream at Norwood, about seven miles from Forest Hills, and seeing *Simulium* larvæ present in small numbers, I took nine specimens from the rocks in order to see whether the species was identical with that common in our streams at Forest Hills. They proved to belong to the same species, namely, *Simulium hirtipes*, but I was much surprised to find that here also the *Mermis* parasite was much in evidence for of the nine specimens taken, six contained the worm. The effects however in these specimens were not so marked as in those found nearer home, for four of the six had turned brown and although the histoblasts were much smaller than is normally the case they were readily visible to the naked eye. Undoubtedly, however, maturation was impossible, for even should the larva manage to pupate it would soon die for want of oxygen, since the respiratory filaments were but half formed.

In a third locality, the Stonybrook Reservation near Forest Hills, a different species of larva was found. Professor Johannsen states that this larva is quite new to him and may be the undescribed larval stage of *S. bracteatum*, which occurs frequently in this State, but as I found only adults of *hirtipes* it was impossible to confirm this supposition. These larvæ were, however, also found to suffer from *Mermis* parasitism, but to a much less extent than those of *S. hirtipes*. The effects on the host were, however, as one would expect, identical with those on *hirtipes*, though the increased size was not so evident.

A large number of larvæ were measured, cut open and the worms removed. It is interesting here to note that even in fixed specimens where the fluid had caused the skin to become opaque, the presence or absence of worms could in all cases be determined with certainty by a glance at the histoblasts. The worm, it should be noted, did not cause the abdomen to swell up or become distorted to any appreciable degree.

A few selected results shown by the examination are appended:

	Length.	Color of Larva.	Histoblasts.	No of Worms.	Size of Worms.
1	10.75 mm.	Gray.	Minute.	12	Av. .75–1 cm.
2	11.50 mm.	Gray.	Minute.	1	2.75 cm.
3	11.0 mm.	Gray.	Minute.	1	3.00 cm.
4	11.0 mm.	Gray.	Minute.	3	1.3, 1.5, 1.5 cm.
5	10.5 mm.	Brownish.	Minute.	1	3.00 cm.
6	9.0 mm.	Gray.	Quite small.	3	.9, 1.3, 1.5 cm.
7	8.5 mm.	Brownish.	Large.	0	———
8	10.5 mm.	Gray.	Large.	0	———
9	8.0 mm.	Brownish.	Large.	0	———
10	7.0 mm.	Brownish.	Large.	0	———
11	8.5 mm.	Gray.	Large.	0	———
12	11.0 mm.	Gray.	Large.	0	———

From this table it will be seen that when there is but one worm in a host it attains a length of about 3 cm. during its parasitic life; that is, roughly about three times the length of the host itself, since the average length of a parasitized larva is 10.5–11 mm. It will also be seen that in one case as many as twelve worms were removed from a single host, and that in this case they all remained small, owing doubtless to insufficient food supply. In other instances, however, where several worms were present in one host it was noticed that one had attained almost to the normal size of 3 cm. whereas the remaining worm or worms were less than 1 cm. in length.

The larval measurements showed that parasitized larvæ average about 11 mm. (Plate III., Fig. 8*a*) whereas mature, and therefore somewhat contracted, larvæ, average some 8–8.5 mm. (Plate III., Fig. 8*b*), though a few are abnormally large.

Life History of the Worm.

As yet nothing is known of this except during the latter part of the life in its host. In all probability the eggs or young worms, are caught as they float down stream by the outspread larval rakes and are swept into the mouth, passing into the alimentary tract through the walls of which they bore their way till they enter the body cavity. It should however be noted that no scars or hypertrophy of the wall of the alimentary tract were noticed in several serial sections made of parasitized larvæ. The alternative hypothesis is that the larval worms bore into their hosts through the thin cuticular covering of the abdomen. That they should be present in the *Simulium* eggs is hardly possible, and this hypothesis can be with safety rejected.

Either of the former hypotheses offers a possible explanation for the variation in development of the histoblasts of parasitized larvæ taken from different streams, for in the case of those taken at Norwood where the discs were of a moderate size, the worms may not have entered the body cavity till the discs were somewhat developed. Then again where one worm has grown to nearly the normal size of 3 cm. whereas other worms in the same host have remained small, it is probable that the large worm entered the body cavity some time before the others.

Figure 9, Plate IV., which is a drawing of a median longitudinal section of a parasitized larva, shows the ventral position of the worm with regard to the alimentary tract, though it will be seen that the mesenteron has been somewhat pushed to one side by the coiled parasite. It will also be seen by comparing Fig. 9, Plate IV., with Fig. 11, Plate III., that the parasitized larva has much less fat body than that of a healthy individual at about the same stage of growth.

Another effect of the host is that apparently the sexual organs do not develop in parasitized larvæ. These are not easily seen in a healthy *Simulium* larva, as they are very small, but they can usually be found in a good series of sections; in sections of parasitized larvæ I have been entirely unable to locate them.

Otherwise the parasite appears to have no effect on the internal organs of the host, with the exception of displacing the spinning glands, the largest portions of which normally lie in the position later occupied by the parasite. Although they may be so displaced as to lie dorsad of the alimentary tract (Plate IV., Fig. 9, *b*) their functioning is in no way impaired, since these larvæ spin quite as many silken threads as do those which are uninfested.

The *Mermis* probably feeds directly on the blood plasma and fatty tissue of the host.

As the larva reaches maturity the contained worm becomes very restless, and if a living larva be placed under the low power of a microscope in a cell slide the movements of the worm can be easily observed. One thus watched for about half an hour was seen to explore the hypodermis of the host, evidently attempting to find a place of exit. The head was kept constantly moving over the internal surface of the abdominal hypodermis and was even sometimes thrust far into the thoracic region. The movements appeared to cause the host great pain, especially when the thoracic region was visited. Finally after the entire worm had been several times twisted round in the body the head was pressed against the junction of the third and fourth abdominal segments and a hole was quickly made through which the worm slowly emerged. The worm, measuring 3 cm., took in all 27 minutes to disengage itself after puncturing the skin of its host. At first the operation was very slow and the constant writhing and turning of the host impeded rather than aided the movements of the parasite. When about 50 mm. were exposed the head was twisted around the body and the remainder of the worm was more rapidly forced out, finally becoming detached from the host in a tightly knotted mass which soon straightened out. The larva meanwhile rapidly shrank, and in about half an hour was dead. The worm apparently can leave the abdomen at almost any point, though the thin junction between two segments is usually chosen. It is probable that the death of most parasitized larvæ is directly due to the escape of the worm, which in all observed cases occurred some time before maturity.

On raising leaves in the bed of a stream a little below a large colony of *Simulium* larvæ it was found that they had under them several of these white worms. This year most of the worms had escaped by the beginning of May, and have since been lost sight of, though while examining a stone microscopically for a second brood of *Simulium* eggs, on May 29, one or two minute worms were seen, which may have been young *Mermis*. No *Simulium* eggs could be observed. At a still later date, August 3, a full grown, though dead, *Mermis* was found under a stone in the bed of the stream.

Percentage of Larvæ Infested.

Several leaves covered with larvæ were taken from a stream in Forest Hills for the purpose of determining the percentage of parasitism.

These larvæ, which represented the species *Simulium hirtipes*, gave the following results: Number of larvæ present 174; number of parasitized larvæ 41. This means that in this particular locality about 23.5 per cent. of the larvæ would be unable to mature on account of the *Mermis* parasite.

The species living in the Stony Brook Reservation was found to be parasitized only to the extent of some 3–4 per cent. A few *S. hirtipes* were also present in the same stream and were parasitized only to the same extent, so it is probable that this smaller attack was not due to the host's belonging to a different species.

Cause of the Retardation of the Histoblasts.

In order to arrive at some definite conclusion as to why the histoblasts of a parasitized larva should not develop normally, it will be of advantage to review briefly some of the theories which have been advanced to explain the normal development of these discs, and at the same time to consider the facts which have been brought to light by the study of several cases now on record in which the discs have developed with abnormal rapidity, thus producing larvæ which possess characters that normally do not make their appearance until the pupal or adult condition is reached. This abnormal condition has been termed "prothetely" by Kolbe (1903).[1] For the abnormally retarded condition as produced in the *Simulium* larvæ by the presence of *Mermis* I should like to suggest the word "metathetely."[2]

The earliest report of prothetely was made by Heymons ('96). In this case larvæ of *Tenebrio molitor* L. were found which, when mature, proved to be abnormally developed as follows:

1. The meso- and meta-thorax possessed expanded lateral portions of the tergites, which resembled the wing-pads of the pupa, though they were not folded under the body as in the latter, but were directed backwards.

2. The antennæ had additional incompletely segmented joints, thus approaching the 11-jointed adult condition.

3. The abdominal tergites were modified so that they resembled the tergites of the pupal abdomen.

These larvæ were raised in the mealworm cultures of the

[1] Προθεῖν, to run before, and τέλος, the completion.

[2] Μεταθεῖν, to run behind, and τέλος, the completion.

Berlin Zoölogical Institute, but no statement is made as to whether conditions during development were quite normal. Heymons concludes his paper by suggesting that the abnormal development described above is due entirely to an accelerated development of the histoblasts, but makes no suggestions as to the cause of the acceleration.

In 1903 Kolbe reported and figured an interesting case of prothetely in the larva of *Dendrolimus pini* L. He received the larva when in its fourth moult, at which stage it had the following characters:

1. The larval antennæ were replaced by elongate antennæ showing simple primary division into about seven segments.
2. The larval thoracic legs were replaced by three pairs of jointed legs possessing all the adult parts, namely, coxæ, trochanters, femora, tibiæ and tarsi.
3. The mouth parts were modified.

Kolbe points out that all these organs were in an immature adult, or true *pupal* form, thus showing, it would seem, that the development was quite normal, but simply accelerated. Winneguth succeeded in breeding from a similar larva an adult which hatched as a small male that was apparently quite normal except for its dwarf size. These abnormal larvæ were produced from an artificially hatched generation kept indoors and from parents which did not hibernate.

Hagen ('72) gives an account of silkworm larvæ obtaining wings before pupation, which condition was accompanied by other abnormalities as follows: The head was small and had two small facetted eyes, and the thorax became modified but the abdomen remained in the normal condition of a larva in the fourth moult. The fore wings were long and narrow and rather more gray than usual, while the hind wings were short and narrow. As this anomaly occurred frequently and was therefore liable to be of economic importance, its causes were investigated by Majoli who came to the conclusion that it was due to the larvæ being kept at a temperature above the normal.

A case similar to that of Heymons was described by Riley ('08) from another coleopterous larva, *Dendroides canadensis*, which was bred by a student at Cornell University.

It will be seen from these cases that in every instance they occurred in artificially reared larvæ, and it is probable that the prothetely was due to an increased temperature, perhaps with the aid of abundant food, in some way hurrying on the development of the histoblasts.

This fact suggests that the histoblasts may be caused to develop, though at a much slower rate than the larval organs until pupation, by some enzyme secreted by the insect and that they can develop only as fast as this stimulant is formed. Its supply thus acts as a regulator. An increased temperature is in most cases advantageous to enzyme action and in this case it is probable that it either causes more of the enzyme to be secreted or stimulates the action of the amount already available.

Dewitz ('05), after numerous experiments on retardation and acceleration of development and pupation arrived at the following conclusions: Development and pupation are caused by enzymes which are not very evident in the early larval stages. They, however, increase with the growth of the larva until its pupation, at which period they are at their maximum strength; they then begin to diminish, till at the end of the pupal period their action entirely ceases. He also states that the enzyme action can be hindered by the presence of another body; and that by obtaining an enzyme of an increased strength before pupation development can be abnormally accelerated.

It is evident that the cells of the histoblasts are caused to develop by a different stimulus from that which causes the development of the larval organs, for in the former case development is very slow during the larval stage, when the latter organs are developing very rapidly, and it is only when these have reached their limit of development at pupation, that the adult organs begin to develop with any rapidity.

This suggests that there may be in the insect body two sets of enzymes which one may term "larval" enzymes and "imaginal" enzymes. These are sufficiently different so that conditions which cause the acceleration or retardation of one of them need not necessarily have any effect on the other. If this be so, one has a probable explanation of prothetely and also, as I shall attempt to show in the following paragraphs, of metathetely.

At first sight one would suppose that the retardation in development of the histoblasts in parasitized *Simulium* larvæ is simply due to a lack of proper nourishment for these organs, since the larva, besides having to supply the requirements of its own developing larval tissues, has also to supply the demands of its fast-growing parasite. This may be true to a certain extent, but later observations, when another parasite is also present, indicate that there must be some other more potent factor whch accounts for this inhibition. This second parasite is a Sporozoön which, owing to the vast numbers in which it occurs in a single host, is far more bulky than the worm and must, one would imagine, make far greater demands on the resources of its host. In this case, however, the histoblasts are usually unaffected in size, though in many cases they are distinctly smaller than normal. Two individuals, however, were seen in which the histoblasts were minute. On dissection it was found that, in each case, a small worm measuring only some 7 mm. was living embedded in the mass of spores, and it was evident that this minute worm was responsible for the retarded condition of the discs, even though it had evidently absorbed very little nourishment. One must, therefore, in all probability look to some *toxin* secreted by the worm as the cause of the inhibition of development in the discs.

The researches of Verson and Bolle, as quoted by Fischer ('06), proved in the case of lepidopterous larvæ that in their early stages their body fluid is alkaline and that this alkalinity decreases as the larva matures. This would suggest that an alkaline condition encourages the growth of larval tissues whereas acidity, or the absence of alkalinity, permits of the development of the adult organs. Hence the histoblasts would develop but slowly till the larvæ are nearing maturity when the decreased alkalinity of the blood allows the "adult" enzymes to stimulate the cells of these discs to rapid division. I have been unable to find any account of the excretions of *Mermis* or of closely related Nemathelminthes, but should they be proved to have an alkaline reaction the probability of the above contention would be very greatly strengthened, for here we should have a case of the alkalinity of the system being maintained in maturing larvæ, and thus preventing the normal, though slow, development of the adult organs, without affecting the larval organs to any great extent, except in so far as they are kept well supplied with alkaline fluids and are thus capable of developing to their utmost extent. This would account for the somewhat larger size of parasitized individuals.

It may be, however, that the *Mermis* does not actually secrete an alkaline substance, but brings about an increased alkalinity in the body of its host by absorbing whatever acids are formed in it. The probability of this being the true condition is increased by the fact that closely related worms live in acid media, such as vinegar or sour paste (*Anguillula aceti* Ehrbg.), which would point to the fact that the worm requires, and absorbs, acids during its development. In either case the effect on the host would be the same in that there is a reduction in the activity of the acids which appears to be essential to the development of the histoblasts. Whether this is the true explanation or not, it is certain that the presence of the worm does have a direct inhibitory effect upon the development of the imaginal organs, but does not have a similar effect on the larval tissues.

A suppression of pupal and adult organs will naturally be of advantage to the parasite for two reasons:

1. Nourishment is not required for building up these tissues and therefore more will be available in the body cavity of the host.

2. The maturity of the host will be deferred thus giving the parasite a longer life, should it require extra time for development. In most of the observed cases, however, the worm killed its host, by emerging before or at about the same time that the uninfested larvæ were pupating.

A similar though less marked case of metathetely due to parasitism by *Mermis* has been described and figured by Mrázek ('08) in the queens of a European ant (*Lasius alienus*). In this case the parasitized larvæ matured and produced adult ants which were normal in all external characters with the exception of a great reduction in the size of the wings. Through the kindness of Professor Wheeler I have been able to examine some similarly parasitized specimens of a closely related American species (*Lasius neoniger*) in order to see whether the development of the legs had been in any way affected but a careful comparison of the measurements of the legs of the parasitized ants with those of healthy specimens failed to reveal any inhibition of their development on account of the *Mermis*, although the wings, which in normal ants measured some 10–11 mm. in length were reduced in the parasitized individuals to 6–6.5 mm.

In the case of *Simulium* larvæ, as before stated, the development of the legs also is inhibited by the presence of *Mermis*, though comparative measurements of the wings and leg histoblasts in healthy and parasitized larvæ show that whereas in the former case the wing histoblast covers about four times the area covered by that of the mesothoracic leg, in parasitized larvæ these histoblasts bear a relation to each other in size of about 2.5 : 1, showing that the wing histoblast suffered a greater inhibition in development than did that of the leg.

A further interesting case of *Mermis* parasitizing ant larvæ was described by Wheeler ('10), in which case the worker larvæ of *Pheidole commutata* were parasitized, and resulted in the adults of such larvæ not only possessing all the normal "worker" characters perfectly developed, but owing to excess of feeding on account of their constant hunger these "worker" larvæ developed, when mature, characters such as the ocelli which are normally only found in the sexual ants.

A Sporozoön Parasite of Simulium Larvæ.

While dissecting out worms from a batch of larva taken on March 30, I chanced to cut open one larva which from the whiteness of the abdomen I took to contain a worm, but was much surprised to find that the body cavity was closely packed with a white substance which had the appearance of cotton wool. A little of this substance, however, when smeared on a slide was seen to be composed of countless organisms as illustrated in Plate V., Fig. 14. My first impression, very naturally, was that these were spermatozoa, which they resemble very closely in general outline. It was, however, very difficult to imagine to what possible organism these spermatozoa could belong. The larva itself could surely not produce them; but if not the larva what then? The only explanation seemed to be that they were formed by one of the worms, which could not then be *Mermis* but must be a new form closely related to *Allantonema* or *Sphærularia*, in the females of which the uterus becomes protruded and is finally many times the size of the original worm.

I naturally visited the stream in order to obtain more specimens suffering from this disease, only to find that a quantity of oil which had been flowing down the stream for some days past had succeeded in killing off all the larvæ in that neighborhood. It was therefore necessary to find a place further up the stream, above the contamination, where the larvæ were living. About half a mile's walk led to such a place, situated under a stone arch, in which the larvæ were present literally in thousands. They formed great masses covering the whole surface of the rocks. An examination of a few rocks soon showed that the *Mermis* was very plentiful here, but it was some time before I found specimens with the curious white abdomens for which I was searching. I chanced, however, to pull up a piece of water weed that was floating in a swiftly running swirl and here I found quite a number of individuals, some showing immense abdominal swellings due to the parasite. On examining a few small specimens I found that the parasite was more numerous among them than among the larger individuals; a closer examination of the rocks showed that these small parasitized individuals were quite commonly scattered among the larger healthy ones. A quantity of material was taken back to the laboratory to be studied on the same lines as that adopted for the *Mermis* parasite.

External Effects on the Larva.

The first effect noticed in badly infested larvæ is the immensely distended abdomen. In some cases as in that illustrated in Plate V., Fig. 12, the apex of this region of the body was three to four times its normal size. Unlike the *Mermis*, this parasite is not confined to the ventral portion of the body but entirely surrounds the caudal extremity of the mesenteron, and small detached colonies were not infrequent in the thoracic region. The Malpighian tubules, however, can usually be seen on the surface of the mass and are plainly visible through the tightly stretched skin, which is quite transparent in places and appears to be on the point of bursting.

The length of the larvæ next claims attention. No larva containing this parasite was found to be exceptionally large; whereas, as before mentioned, many were extremely small, measuring some 2.75 or 3.5 mm. at a time when all normal larvæ measured some 9 mm. or more. This may possibly require a similar, though opposite, explanation to that suggested on page 281 to account for the large size of the individuals affected by the *Mermis*.

In confinement these small specimens were extremely restless, they were continually twisting about and moving over the sides and bottom of the jar with a peculiar jerky movement. The larger, and more heavily parasitized, larvæ on the other hand were more sluggish though not more so than the healthy larvæ.

The effect of these parasites on the histoblasts is very hard to state with any certainty. Fig. 13 is an illustration, made with a camera lucida, of the histoblasts of a nearly mature individual. In this case the parasite was confined to the abdomen. It will be seen that the discs are but half developed (compare with Fig. 6). In other cases, however, the development of the discs was not, so far as could be seen, affected in any way till pigmentation of the respiratory filaments commenced. Then it was noticed that the entire histoblast only turned a slate gray color instead of blackening at the apex of the filaments and finally over the complete disc. In other cases, however, the discs entirely blackened in a perfectly normal manner.

When colonies of parasites are located in the thorax, as is not infrequently the case, the histoblasts are materially decreased in size, while in the very small specimens development appeared to have been arrested. This is hardly surprising. My general conclusions were that the parasite affected the histoblasts but little, though the larvæ rarely advanced so far toward maturity that the respiratory filaments became pigmented. It should be noted that the power of spinning silk was in no way affected by the presence of this parasite.

Nature of the Parasite.

While visiting other localities I made numerous observations on the larvæ to be found in the streams, and was much surprised to find that species of the parasite were extremely common and that in almost every brook visited some of these conspicuously distorted individuals were to be found. On making microscopical examinations I observed that the organisms taken from different localities varied very much in form and apparently represented different species. A list of these different forms and their hosts is appended:

1. Ovoid bodies bearing a "flagellum"-like organ varying in length from about that of the "body" to three times its length (Plate V., Fig. 14). Host: *Simulium hirtipes.* Habitat: Forest Hills and Blue Hills, Mass.

2. Bi-annulated ovoid bodies bearing a "flagellum," which is never much longer than the "body" (Plate V., Fig. 15). Host: *Simulium* species undescribed. Habitat: Stony Brook Reservation, Mass.

3. Ovoid bodies having the "flagellum" replaced by a transparent flattened disc (Plate V., Fig. 16). Host: *Simulium* species undescribed. Habitat: Stony Brook Reservation, Mass.

4. Simple ovoid bodies destitute of all appendages (Plate V., Fig. 17). Host: *Simulium* species undescribed. Habitat: Stony Brook Reservation and Blue Hills, Mass.

The following characters were common to all of these organisms:

1. They were all similar in size.

2. Under the highest power of the microscope they had a faint olive greenish tinge.

When in a fresh condition absolutely no internal details were visible and in specimens which had been killed, fixed and stained very little more could be seen. In many of the first or "spermatozoid" type a darkened central area indicated the presence of a very large nucleus, while a smaller dark spot just before the base of the flagellum might be taken for the nucleolus.

Many of the "simple ovoid" type, although but slightly staining, showed a great shrinkage of the internal substance; otherwise nothing could be seen in them. The specimens with a flattened disc, and those bearing two annuli and a flagellum, also showed practically no differentiation. Methyl green and various haematoxylin combinations were tried without success.

Besides these various forms, no two of which were observed to occur in the same individual, another form of cell (Plate V., Fig. 18) was found in much smaller numbers, but possibly in some way connected with them, for it was seen in association with each form, but was not found in healthy larvæ. This cell, which varied much in diameter from but little more than the numerous "spores" to three or four times the length of their longest axis, was apparently globular in form, and in a fresh state was very transparent and could only be discerned with difficulty. The following characters, however, were seen. The substance of the cell was finely granular, and often contained a number of large transparent globules. When fixed and stained the only differentiation was that of a large dark mass, apparently the nucleus (Plate V., Figs. 18, *a* and *b*). In some cases these cells seemed to be dividing (Plate V., Fig. 18, *c*).

In the face of all these diverse types of cell, which live in precisely the same way, it is evident that the forms first found are not spermatozoa, and further the fact that the flagellum is replaced in one "species" by a flattened disc eliminates the possibility of their being *Flagellates*. It is therefore probable that one must look to the *Microsporidia* among the *Sporozoa* as the group to which these bodies belong. It is further noticed that many of the forms are very similar to those met with in the genus *Glugea* to which the well known *pébrine* disease (*G. bombycis*) of silk-worms belongs. One must therefore conclude that it is a pebrine-like disease, which is killing off a high percentage of the Simuliid larvæ in the neighborhood of Boston, though on account of the vast difference in structure and mode of life of the two hosts the life history of the parasite in the *Simulium* larvæ is very unlike that described by Pasteur ('70) and Stempell ('09) in their work on the disease in silk-worms.

Life History of the Parasite in Its Host.

As in the case of *Mermis*, I know very little about this, since I did not discover it till the final stages of the larva were being approached, and in every instance but one it was apparently in exactly the same condition, namely, the "spores" were all formed and were simply awaiting the death of their host and its resulting decomposition to escape into the water.

The one exception, however, was that of a larva found on April 17. It was one of the first discovered, in the abdomen of which could be seen, with a dissecting lens, large white bodies floating in the blood plasma. On dissection these bodies proved to be rounded masses of flagellate spores, a few of which had become disengaged and were apparently moving about by their own impetus in the blood plasma. As, however, I have never on any subsequent occasion observed such a movement among these spores I am inclined to believe that this was simply a Brownian movement, which I have distinctly recognized in later examinations. Though I searched carefully for other larvæ with parasites in a similar stage I was unsuccessful.

In serial sections of parasitized larvæ similar effects to those exhibited when *Mermis* is present, are noticed in that the fat-body is much reduced (Plate IV., Fig. 10) and the sexual organs could not be detected, whereas the spinning glands and musculature apparently remained unaffected. The parasite probably enters the host through the alimentary tract for in all cases of infected larvæ it was found that the mesenteron was distorted in one or more places showing distinct hypertrophy as if the cells had been badly irritated but had healed over again often permitting the spores to pass through into the body cavity (Plate IV., Fig. 10, *e*). In one case a small colony of "spores" was found inside the alimentary tract, but it is quite possible that these had been recently taken in with the water, as parasitized larvæ were constantly dying in the colony, and any material, floating in the water such as liberated "spores," would be caught by the cephalic fans of still living larvæ.

In all recorded cases of Microsporidia parasites it has been noticed that the "spores" live, at least during their early stages, inside some body tissue of the host. An example of this is to be found in pébrine of silk-worm larvæ, in which case the spinning glands are the main seat of attack. It must be borne in mind, however, that an attack upon these organs in the larva now under consideration would, in all probability, result in the early death of the host, for it is only by means of the much strengthened silk threads that the larva is enabled to maintain in rapid streams the perpendicular position which is essential to obtaining food, while at the same time the larva has to depend to a large extent on these threads for retaining any foothold on the rocks, for when moving its position, the adhesive disc of the proleg frequently loses its grip and the larva is washed clear of the rock. Very rarely, however, the anchoring threads, which are always present, break so that the larva is able slowly to draw itself once more to its support. Sections of the *Simulium* larva disclose the fact that there are very few other tissues in the body. The muscular system is much reduced, and the only tissues available for attack without rapidly killing the host are the fat-body and the sexual organs, and I am under the impression that it is the latter which are usually the original seat of attack. In frontal sections of a very young larva taken during March, only one testis could be found, but on the other side of the body a small mass of minute cells, taken at the time for small œnocytes, was situated a little back of the normal position of the missing testis. The cells are too small to show any structures but it is possible that this is a diseased testis to which the spores made their way as soon as they entered the body cavity. Later sections also show traces of a very thin membrane investing the mass of "spores."

I kept a large number of diseased larvæ in running water, hoping to see some of them pupate, but in every case they died, and soon liberated the spores, whereas many of the healthy larvæ, which were approaching maturity, pupated in captivity. It is thus evident that heavily parasitized larvæ never pupate, but die in the stream, liberating their countless spores in the water. What happens to these spores I have been unable to ascertain. Larvæ were allowed to die in distilled water and the liberated organisms were examined at intervals, but though they strongly resisted decomposition they never showed signs of movement or altered condition. Sections of pupæ and adults obtained from badly infested localities failed to reveal any cases of the disease being carried beyond the larval stage. It is therefore probable that the disease is not hereditary as is the case with pébrine, and as before stated it was seen that the presence of this parasite, in every case examined, apparently resulted in castration of the host. It is, however, possible that among the vast numbers of larvæ a few were only slightly parasitized and did not have their sexual organs entirely destroyed. Such individuals should be capable of pupation and might in that way carry the disease over as in the case of pébrine. An examination of several hundreds of larvæ did not reveal one in which such a condition was possible.

Were there overlapping generations of larvæ the maintenance of the parasite could be more easily understood, but the spring brood pupated and hatched during the first half of May, and there are not at the time of writing any signs of more eggs being deposited on the rocks. It thus seems that there must be a secondary host in which this parasite passes the summer.[1]

The Percentage of Infested Larvæ.

The number of infested larvæ varied to a great extent in different streams. In the part of the stream in Forest Hills where the parasites were first noticed, less than 1 per cent. of the larvæ were parasitized, half a mile further up the stream a little under 40 per cent. were infested, while in the Stony Brook Reservation where two types of "spores" were present among the parasitized larvæ, between 70 and 80 per cent. of the individuals were to be seen with the immensely distended and whitened abdomens which proclaimed them to be suffering from this fatal disease.

It will thus be seen that should this disease together with the previously described *Mermis* parasite, prove to exist as abundantly in other localities as it has during the past spring in the vicinity of Boston it must be of considerable importance in the natural control of the black flies which are such an annoyance to man and beast, especially in more tropical regions, and if the supposed secondary host of the Sporozoön does not prove to be a fish or animal of any value it should be possible to infect streams where Simuliid larvæ breed, and diminish their numbers very largely without the danger of poisoning the fish by applying oil or other substances to the water.

Summary.

Simulium larvæ, which are found in vast numbers in small rapid streams around Boston, are seen to feed by standing per-

[1] I have since July 31 noticed isolated specimens of a different species of larva in one of the streams where the parasite abounded in the spring brood, but have not found any in which there were signs of the parasite being present.

pendicularly on rocks to which they are attached by a strong anal adhesive disc, and kept in position by silken threads secreted by the salivary glands. While thus anchored they spread out a pair of cephalic fans which act as strainers and collect small particles of food from the water. The head capsule is moulted independently of the body cuticle and exposes a new capsule which is at first white with a few dark spots on the vertex, but which rapidly becomes uniformly darkened all over. The thorax bears unusually well defined and large histoblasts of the imaginal wings, halteres and legs, and also on either side a histoblast of the pupal respiratory filaments, which by turning black when the larva is mature becomes very conspicuous at this stage of growth. The larvæ are infested by two parasites, namely a *Mermis* and a Sporozoön, both of which live in the body cavity.

The *Mermis* does not affect the larval development to any extent, except by slightly increasing its size, but it inhibits the development of the histoblasts to such an extent that pupation becomes impossible.

The embryo worms are probably caught by the cephalic fans of the larvæ and pass into the alimentary tract, through the walls of which they bore and live in the body cavity of the host till the latter matures. They then rupture the abdominal cuticle and pass into the water where they live a free life under stones in the bed of the stream. The number of worms contained by a single larva is usually only one, but as many as twelve have been found. A single worm measures 3 cm., which is about three times the length of the host. In some streams 25 per cent. of the larvæ were infested with this parasite. Parasitized larvæ never pupate, but are killed by the worms when they escape.

The retardation in the development of the histoblasts is the opposite condition to that met with in prothetely which is usually caused by keeping larvæ at an abnormally high temeprature. This probably results in an increased supply of the enzymes which cause these histoblasts to develop. The *Mermis* apparently excretes some substance which lessens the supply or action of these enzymes and leads to metathetely.

The Sporozoön parasite occurs in several forms in different localities. All these forms, however, live in the same way and appear to be related to the pébrine disease of Lepidoptera. The body, especially near the apex of the abdomen, becomes much distorted and swollen on account of its interior being closely packed with a white wooly material which on dissection is seen to consist of countless "spores" of minute size. Such parasitized larvæ are usually rather smaller than healthy individuals, but the histoblasts do not appear to be much affected. The parasite apparently enters the body cavity in the same manner as that described in the case of *Mermis*. Evidence of this is seen in a hypertrophied condition of parts of the mesenteric wall. From here it seems to pass to one or both of the sexual organs which are destroyed and become the nuclei for the great mass of spores which eventually fills the abdomen. The parasitized larvæ in this case also were never observed to pupate but died when mature. The spores are liberated by a rupture of the abdominal wall soon after the death of the host and pass into the water, after which stage they have not been seen. Up to 80 per cent. of the larvæ in some streams were found to contain large masses of this parasite but no cases of slightly parasitized larvæ were observed. There has been no second brood of *Simulium* larvæ this year, so it would seem that if the parasite is to appear next year there must be a secondary host in which the summer is passed.

Postscript.

The foregoing account of the Sporozoid parasite of *Simulium hirtipes* was very kindly reviewed for me by Professor G. N. Calkins, of Columbia University. From mounted specimens of the spores sent him he confirmed my opinion that these represent some species of Myxosporidea, and drew my attention to a paper by Louis Léger ('97), which I had overlooked because of its not being catalogued under *Simulium* in the cards of the "Concilium Bibliographicum" in the Cambridge library. In this paper a new species of *Glugea* parasitizing the larvae of the European *Simulium ornatum* is described as *G. varians*. The notes on this species are in brief as follows: The abdominal region of the infested larva is greatly distended and contains large masses of a free parasite in the form of opaque, milky white, irregular sacs.

Some larvæ contain but one mass whereas others contain two to four. The muscles are unaltered, but the fat body is much reduced. The alimentary canal always appears to be contorted. In only one case had the Microsporidea failed to sporulate and they then formed a swelling on the external intestinal surface. The sacs contain countless ovoid, refractive spores, which, when treated with iodine, show a filament 15–20 times as long as their longest diameter. Spores of two sizes are present, the smaller measuring 4–5μ, the larger 8μ. In a subsequent note written in collaboration with Hagenmueller ('08) Léger states that the spores are sometimes present in a polysporic and sometimes in an octosporic arrangement. These authors also refer to a similar parasite in the larvæ of *Tipula gigantea*.

From the foregoing notes it will be seen that the disease which occurs in *S. hirtipes* is very similar in its main features to that described by Léger, and I do not hesitate to regard the organism responsible for its occurrence as a closely related form. Professor Calkins is inclined to consider the various forms I have described as belonging to a single species. For this I would propose the name *Glugea polymorpha* sp. nov. Future investigation, however, will quite possibly show that the various forms occurring in different localities are not all representatives of the same species, since numerous dissections of diseased larvæ showed that certain types of spores were peculiar to different localities even though present in two different species of *Simulium* larvæ.

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Explanation of Plate I.

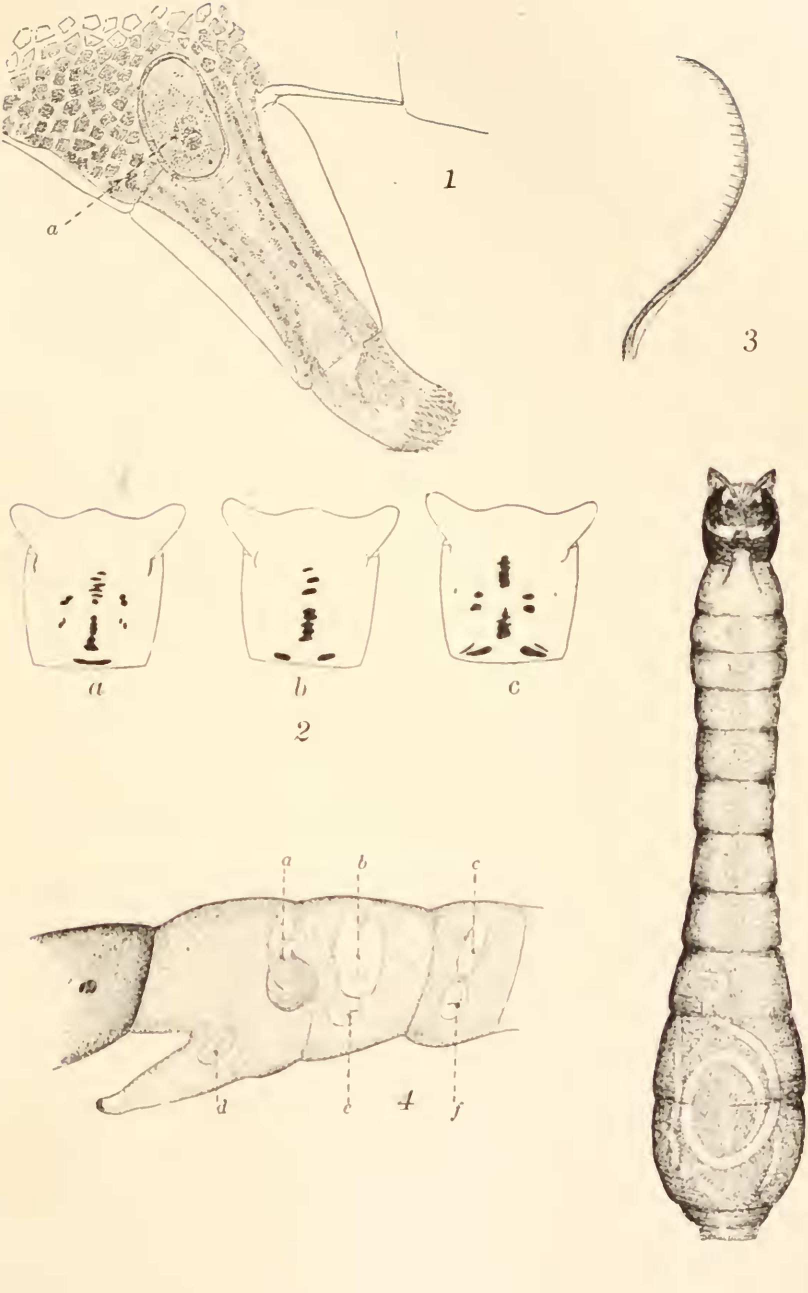
Fig. 1. Prothoracic leg of a *Simuliid* larva showing the histoblast (*a*) at an early stage of development.

Fig. 2, *a*, *b* and *c*. Types of spotting on the head of a recently moulted larva of *Simulium hirtipes*.

Fig. 3. A single rake from the cephalic fans.

Fig. 4. Profile of thorax of a half-matured larva showing the histoblasts. *a*, respiratory organ histoblast (pupal organ); *b*, wing histoblast (adult organ); *c*, halterer histoblast (adult organ); *d*, *e* and *f*, pro-, meso- and metathoracic leg histoblasts (adult organs).

Fig. 5. Ventral view of a *Simulium* larva with *Mermis* parasites in situ.



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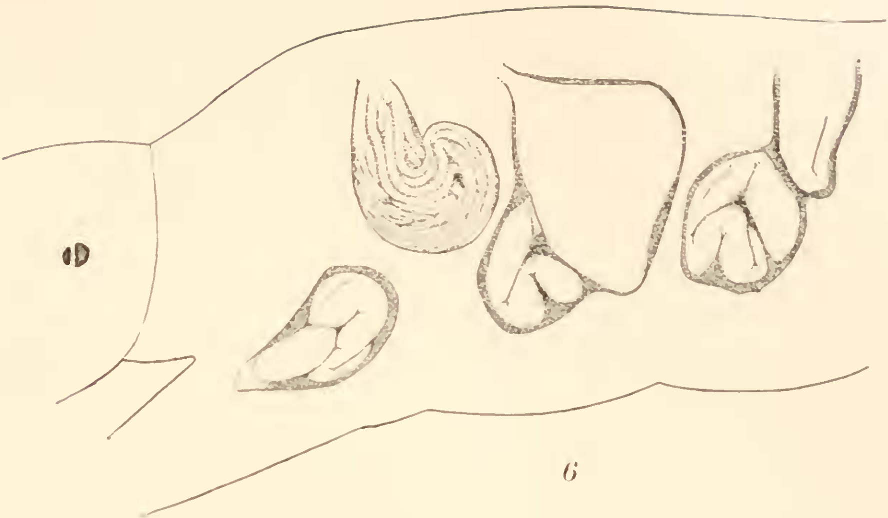
Explanation of Plate II.

Fig. 6. Histoblasts of a healthy full-grown larva measuring 10.5 mm.

Fig. 7. Histoblasts of a larva parasitized by *Mermis* measuring 10.5 mm.



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Explanation of Plate III.

Fig. 8*a*. Average size full-grown parasitized larva of *Simulium hirtipes* measuring 11 mm.

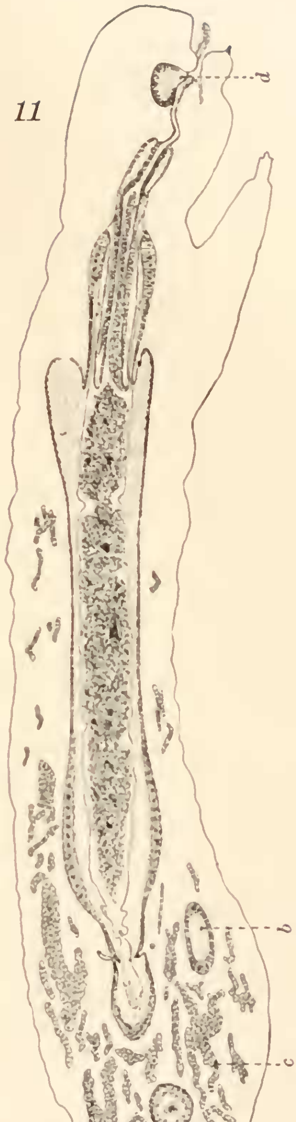
Fig. 8*b*. Average size mature larva of *Simulium hirtipes*, measuring 8.5 mm. showing darkened pupal histoblast, *a*.

Fig. 11. Median sagittal section of a half-grown healthy larva to show the alimentary tract. *b*, the normal position of the spinning glands, which extend backwards from the pharynx on either side latero-ventrally to the alimentary canal, doubling back on themselves at about the point *b*, where one has been cut in cross section; *c*, the normal quantity of fat body which increases still more as maturity approaches; *d*, one of the pharyngeal glands. (This is not seen in an exact median section, as the two glands are narrowly separated medially.)

8 a

8 b

11



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Explanation of Plate IV.

Fig. 9. Median sagittal section of a larva parasitized with *Mermis* sp. to show the somewhat displaced alimentary tract, and *a*, the coiled up worm; *b*, the displaced spinning gland, a short longitudinal section of which has been cut; *c*, the much reduced fat-body; *d*, one of the pharyngeal glands.

Fig. 10. Median sagittal section of a larva parasitized with a sporozoön to show *a*, the mass of spores, a few of which are shown enlarged; *b*, the spinning gland; *c*, the much reduced fat body; *d*, one of the pharyngeal glands, and *e*, the somewhat distorted wall of the alimentary tract.

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Explanation of Plate V.

Fig. 12. Dorsal view of a *Simulium* larva with *Sporozoid* parasites in situ. Compare with Plate I., Fig. 5, which is normal in shape.

Fig. 13. Histoblasts of a larva parasitized with the *Sporozoa* measuring 9 mm. Compare with Plate II., Fig. 6.

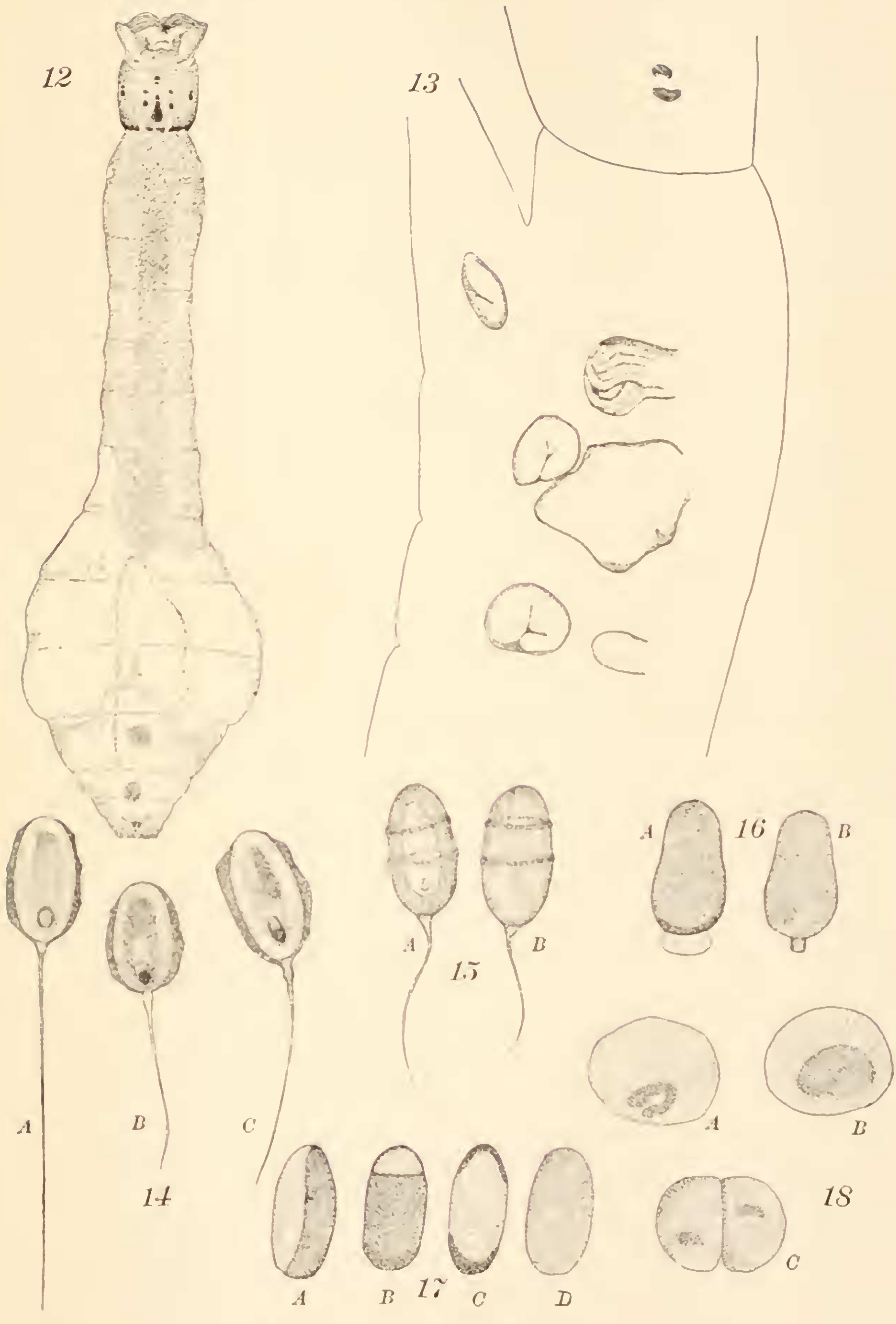
Fig. 14, *A*, *B* and *C*. Simple "flagellate" type of parasite, ×4,000.

Fig. 15, *A* and *B*. Annulate "flagellate" type of parasite, ×4,000.

Fig. 16. Type of parasite with "flagellum" replaced by a flattened disc, *A* showing surface view of disc, ×4,000; *B* showing side view of disc, ×4,000.

Fig. 17, *A*, *B*, *C* and *D*. Simple ovoid type of parasite, ×4,000.

Fig. 18, *A*, *B* and *C*. Other bodies found in small numbers among the "spores," ×4,000. Many are larger in proportion than those illustrated. *C* shows one apparently dividing.



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BIOLOGICAL BULLETIN

THE PERSONAL EQUATION IN BREEDING EXPERIMENTS INVOLVING CERTAIN CHARACTERS OF MAIZE.[1]

RAYMOND PEARL.

In the summer of 1908 some experiments in the cross-breeding of certain types of maize were begun by the writer and his former colleague, Dr. Frank M. Surface. The present paper has to do with a part of the results obtained by crossing a white sweet variety (♂ parent) with a yellow dent variety (♀ parent). Both varieties used were "pure," in the sense that each bred true to the general type to which it belonged. The history of the sweet variety used has been detailed in another place[2] and need not be repeated here. The important thing to be noted at this time is that in its whole history this sweet corn used in the cross breeding experiments had never been known to produce any but *sweet* (sugary) kernels of an exceptional degree of *whiteness*.[3]

The dent corn used in the experiments was also of known history. A discussion of its history, and of the characteristics of the corn has been given elsewhere.[4] The essential point to be noted here is that during a long period of years it has never produced anything except *starchy* kernels of a deep orange *yellow* color when ripe.

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 29.

[2] Pearl, R., and Surface, F. M., "Experiments in Breeding Sweet Corn," Me. Agr. Expt. Stat. Ann. Report for 1910, pp. 249–307.

[3] A pure chalky white or, put in the other way, the entire absence of yellow color, is an absolute essential of a high grade of corn from the packer's standpoint. The sweet corn here under discussion is regarded by expert packers as an exceptionally fine strain for their purpose.

[4] Pearl, R., "The Mendelian Inheritance of Certain Invisible Chemical Characters in Maize." *Zeitschr. f. Abst.- u. Vererb.-lehre*, Bd. VI., 1911.

The general results which follow the crossing of a yellow dent (♀) with a white sweet (♂) maize are well known. Yellowness of endosperm is dominant over "whiteness" of endosperm, and "starchiness" over "sweetness." Consequently the F_1 kernels are externally indistinguishable (in fact as well as in theory) from those of the pure yellow dent parent. These F_1 kernels planted give rise to plants bearing ears of which each should have four distinct kinds of F_2 kernels which ought, by theory, to occur in the simple dihybrid ratio, 9 yellow dent, 3 white dent, 3 yellow sweet, 1 white sweet.

The present experiments[1] entirely confirm in all essential respects this general Mendelian result. Certain novel points arose, however, in the course of the work, which led to the present investigation. These points may now be considered.

A large quantity of ears bearing F_2 kernels was raised. These ears were well matured. This was indicated both by their appearance and by the way the seed from them germinated. One of the assistants in the laboratory, Miss Maynie R. Curtis, undertook the sorting and counting of these F_2 kernels on an extensive scale. In this work the following situation immediately developed and was called to the writer's attention. While *in general* the F_2 kernels fell without any doubt or difficulty into the four classes or categories, yellow starchy, white starchy, yellow sweet and white sweet, yet there were a number of kernels on each ear that were extremely difficult of classification. These kernels were, in short, *intermediate* in respect to their external visible *somatic* characters, and might, in the individual case, be put with equal propriety into either of two classes. Into which class such an intermediate kernel would actually be put plainly depended upon the personal bias of the observer, rather than upon any peculiarity of the kernel itself. This result appeared to be of enough interest and potential significance to warrant a more extended and thorough investigation of the matter. The present paper deals with the results of such a study.

[1] A detailed description of the conditions and manner of these experiments has been given elsewhere (Pearl, *loc. cit.*) and need not be repeated.

Statement of Problems and Plan of Investigation.

The problems with which this work is concerned may be summarily stated as follows:

1. To what extent is the personal equation of the observer a significant factor in the Mendelian ratios described for simple experiments with cross-bred maize? In other words, how closely would the different individuals of a group of competent biological observers agree in their classification and count of the *same* F_2 material from a maize cross involving such relatively simple and easily judged unit characters as color of endosperm, or chemico physical character of endosperm (starchy or sweet)?

2. Does somatic "intermediateness" in maize imply gametic "intermediateness"? In other words, do F_2 kernels which are intermediate *somatically* give rise to any different sort of progeny when planted than do kernels which belong clearly and indubitably to one or another of the well-defined *gametic* classes in F_2? If they are true "blends" in the Galtonian sense, they would certainly be expected so to do. If, however, they merely represent a phenomenon essentially like the incomplete or partial (somatic) dominance so frequently observed in Mendelian work, it would be expected that their progeny would differ in no essential particular from that obtained from somatically non-intermediate kernels having the same gametic constitution.

To test these questions the following plan was devised: Four ears bearing F_2 kernels were taken quite at random from a lot of about two bushels of such ears, which in turn was a random sample of a whole crop which included a much larger number of bushels. Each of these four ears was given an arbitrary number and was separately shelled, great care being taken to see that no kernels were lost. All the kernels from each ear were preserved together in a bag (or box). The shelling was done in the writer's laboratory in the presence of several workers, so that there can be no question whatsoever, that all of the kernels in each one of the four parcels originally grew upon the same ear.

Three of the ears so dealt with (Nos. 8, 9 and 10) were normal in every respect. Ear No. 11 was slightly abnormal in the respect that a fungus had attacked some of the grains, giving them a slight pinkish tinge in addition to their own proper color. This was especially noticeable in the case of the "white" sweet grains of the ear, because in a mature, dry sweet corn kernel "white" means merely the absence of any color (yellow or other). The grain is translucent and "not colored." Any extraneous color such as that arising from a fungus attack will be the more evident. The same considerations apply to the white starchy kernels, except that here the starch of the endosperm gives the grain a positive white color.

The kernels from each of the four ears having been separately shelled and preserved as described, fifteen persons (including the writer) were asked to sort the kernels of each ear into the four categories, yellow starchy, white starchy, yellow sweet and white sweet, and then count and record (on blanks provided for the purpose) the number of each sort found. Four small vials containing *typical* kernels of each sort were given to each observer as comparison samples. The only instructions given the observers were:

1. To sort and count the material *independently*.
2. To open and handle only one parcel of seed at a time.
3. Not to lose a kernel.
4. To count correctly, *i. e.*, to make sure that the total numbers of kernels counted tallied with the total numbers in the parcel, which numbers were set down on the blank for each ear.

Especial pains was taken to insure that no observer (with the exception of Nos. VI., VII., VIII. and XI.) should know, in advance of his count, the nature of the experiments which gave origin to the material, or the expected Mendelian ratio between the several classes of kernels. No observer[1] was, of course, allowed to see the results of the counts by others until after his own had been completed. In short every effort was made to insure in all possible ways that the counts tabled should be the unprejudiced, unbiased, independent and purely objective statements of the opinions of a group of competent biological observers as to the proper classification of the F_2 kernels from these four ears of maize.

We may next consider the observers who took part in this work. At the outstart the writer wishes to express his indebted-

[1] With the single exception of No. XI., and in this case it was some *months* later that his own counts were made.

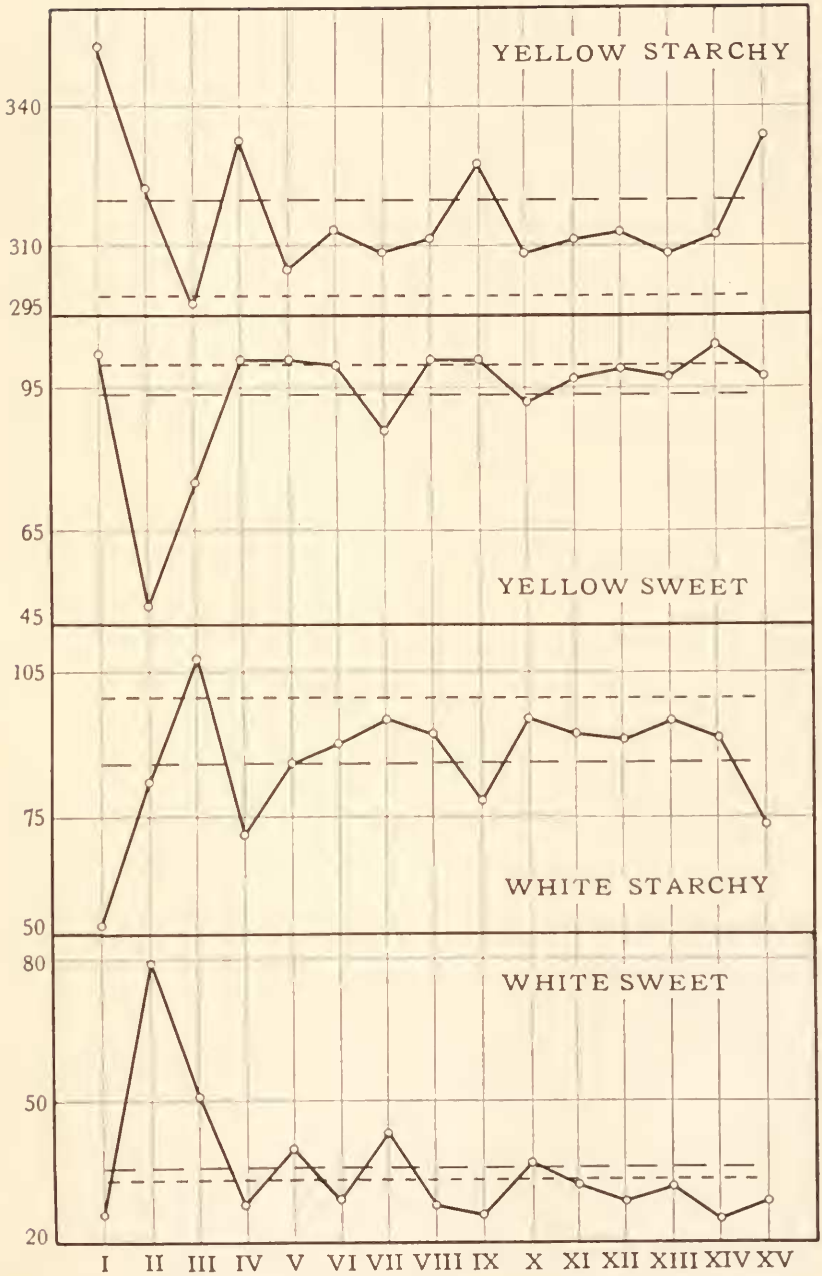

FIG. 1. Diagram showing the count of the different observers of each of the four classes of kernels for ear No. 8.

ness to all of those who coöperated in the investigation, and his appreciation of the painstaking interest and care given to the sorting and counting by all. Table I. gives the name, academic degree and official position of each of the coöperating individuals. For convenience of reference in the paper each observer has been assigned a Roman numeral.

Table I.

List of Observers Coöperating in the Present Study.

No.	Name.	Academic Degree.	Official Position.
I.	W. J. Morse.	M.S.	Plant pathologist, Maine Experiment Station.
II.	C. E. Lewis.	Ph.D.	Associate plant pathologist, Maine Experiment Station.
III.	G. E. Simmons.	M.S.	Professor of agronomy, University of Maine.
IV.	M. E. Sherwin.	M.S.	Assistant professor of agronomy, North Carolina College of Agriculture.
V.	Wallace Craig.	Ph.D.	Professor of philosophy, University of Maine.
VI.	Raymond Pearl.	Ph.D.	Biologist, Maine Experiment Station.
VII.	Frank M. Surface.	Ph.D.	Biologist, Kentucky Experiment Station.[1]
VIII.	Maynie R. Curtis.	M.A.	Assistant in biology, Maine Experiment Station.
IX.	Lottie E. McPheters.	—	Computer, Maine Experiment Station.
X.	Frank Pearl.	—	Farmer and practical corn breeder.
XI.	W. Johannsen.	M.D.	Professor of plant physiology, University of Copenhagen.
XII.	P. Boysen Jensen.	Ph.D.	Instructor in plant physiology, University of Copenhagen.
XIII.	Jenny Hempel.	M.Sc.	Assistant in plant physiology, University of Copenhagen.
XIV.	Gerda Dohlmann.	—	Assistant in plant physiology, University of Copenhagen.
XV.	Gilman A. Drew.	Ph.D.	Professor of Biology, University of Maine.

Certain points regarding this list of coöperators need to be discussed. In the first place it is obvious that any one of them (with the possible exception of X.) might in the ordinary course of his work carry out a Mendelian experiment with maize, either independently or in coöperation with someone else. If this were done and the results published they would certainly be accepted by the biological public as a precise and true statement of the facts regarding the material which was in the experimenter's hands. That is, if any worker in this list published a statement that a Mendelian experiment which he had conducted with

[1] At the time this work was done: Associate Biologist, Maine Experiment Station.

maize led to a ratio of, for example, 759 : 243 : 252 : 90 this statement would not be doubted or questioned.

In the second place it is worth while to consider the training, or lines of work with which these 15 observers have had to do. Of six (Nos. I., II., XI., XII., XIII., XIV.) the training and work has been primarily *botanical*. Four of these (the Danish group,

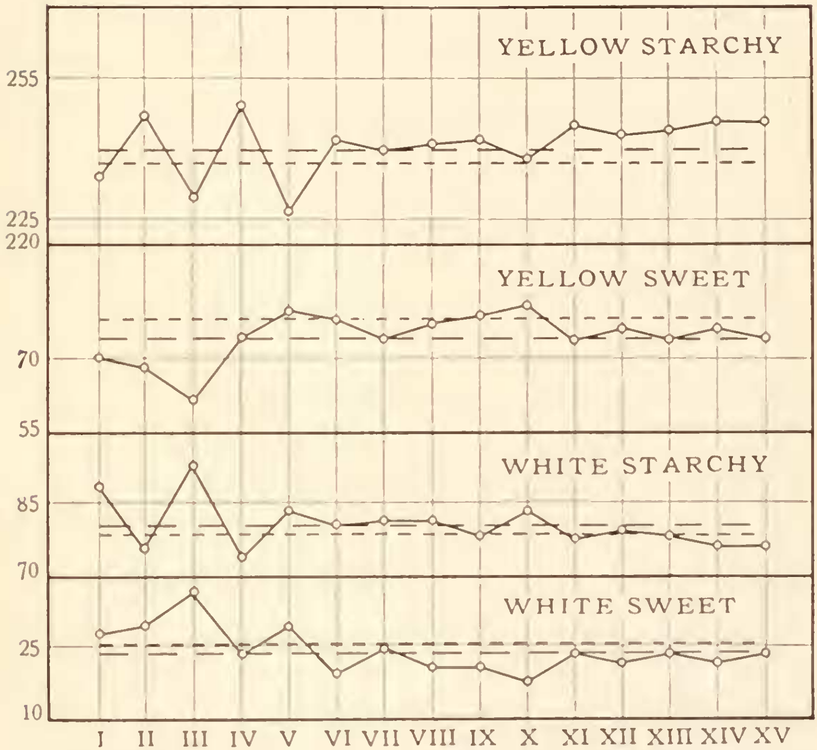

Fig. 2. Diagram showing the count of the different observers of each of the four classes of kernels for ear No. 9.

Nos. XI. to XIV. inclusive) have had particularly to do with the data of experimental plant breeding, in connection with the brilliant and fundamental researches of Professor Johannsen. The training and special field of work of five (Nos. V., VI., VII., VIII. and XV.) of the observers has been *zoölogical*. Of these five three (Nos. VI., VII. and VIII.) have had experience with the data and methods of investigation in experimental breeding.

TABLE II.

SHOWING THE CLASSIFICATION OF THE KERNELS OF EAR NO. 8 BY THE DIFFERENT OBSERVERS.

Observer.	Classes of Kernels.					
	Yellow Starchy.	Yellow Sweet.	White Starchy.	White Sweet.	Total Starchy.	Total Sweet.
Mendelian Expectation.	299.25	99.75	99.75	33.25	399.00	133.00
I.	352	102	52	26	404	128
II.	322	49	82	79	404	128
III.	298	75	108	51	406	126
IV.	332	101	71	28	403	129
V.	305	101	86	40	391	141
VI.	313	100	90	29	403	129
VII.	308	86	95	43	403	129
VIII.	311	101	92	28	403	129
IX.	327	101	78	26	405	127
X.	308	92	95	37	403	129
XI.	311	97	92	32	403	129
XII.	313	99	91	29	404	128
XIII.	308	97	95	32	403	129
XIV.	312	104	91	25	403	129
XV.	333	97	73	29	406	126
Totals.	4,753	1,402	1,291	534	6,044	1,936
Means.	316.87	93.47	86.67	35.60	402.93	129.07

Another of the five (No. V.) adds to the special training of the zoölogist that of the philosopher and psychologist, which by traditional standards, at least, ought to aid in the development of a discriminative judgment. The training of two of the observers (Nos. III. and IV.) has been agricultural. Further, both of these men belong by birth, early life and education to the "corn belt" section of the country, and are thoroughly and intimately familiar with maize. They have had experience in corn judging, which demands the appreciation of very small differences in ear characters. Observer No. X., while not a scientific student of breeding, has had successful practical experience in corn breeding, and is a careful observer. Observer No. IX. has been specially trained in biometric work in the writer's laboratory and has had considerable experience in measuring, sorting small variations out of mixed material, and similar work.

RESULTS.

I.

The results of the counts of the four ears by the different observers are set forth in Tables II. to V. inclusive. Each of

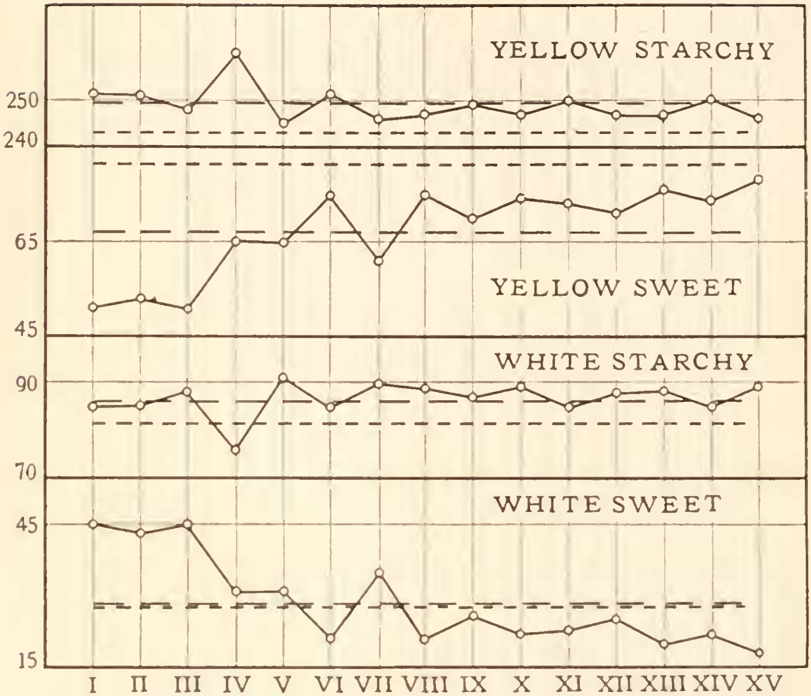

Fig. 3. Diagram showing the count of the different observers of each of the four classes of kernels for ear No. 10.

these tables is arranged as follows: Columns are given for the four different classes of kernels, yellow starchy, yellow sweet, white starchy and white sweet. Also columns are given for total starchy and total sweet. The first row of each table shows the Mendelian expectation for each class. The following lines show the distribution of the kernels as reported by each of the fifteen observers.

The data for the color classes given in these tables are shown graphically in Figs. 1 to 4 inclusive. One of these diagrams is devoted to each of the four ears used in the study. Each figure gives the plotting of each observer's count of the four classes of kernels. The Mendelian expectation is plotted in each case as a dotted straight line and the mean of the results of the different observers as a straight line of dashes.

From these tables and diagrams we note the following points:

TABLE III.

SHOWING THE CLASSIFICATION OF THE KERNELS OF EAR NO. 9 BY THE DIFFERENT OBSERVERS.

Observer.	Classes of Kernels.					
	Yellow Starchy.	Yellow Sweet.	White Starchy.	White Sweet.	Total Starchy.	Total Sweet.
Mendelian Expectation.	237.33	79.11	79.11	26.37	316.44	105.48
I.	234	71	89	28	323	99
II.	247	69	76	30	323	99
III.	230	62	93	37	323	99
IV.	249	75	74	24	323	99
V.	227	81	84	30	311	111
VI.	242	79	81	20	323	99
VII.	240	75	82	25	322	100
VIII.	241	78	82	21	323	99
IX.	242	80	79	21	321	101
X.	238	82	84	18	322	100
XI.	245	75	78	24	323	99
XII.	243	77	80	22	323	99
XIII.	244	75	79	24	323	99
XIV.	246	77	79	22	323	99
XV.	246	75	77	24	323	99
Totals.	3,614	1,131	1,215	370	4,829	1,501
Means.	240.93	75.40	81.00	24.67	321.93	100.07

1. For no one of the ears is there entire agreement among all the observers as to the number of kernels falling in any one of the color classes. There is entire agreement among the observers as to the total number of starchy and sweet kernels in the case of two ears (Nos. 10 and 11), leaving out of account the loss of one starchy kernel from ear No. 10 between the time when this ear was counted by observers X. and XI. In the case of the other two ears (Nos. 8 and 9) there is some disagreement as to the number of starchy and sweet kernels. In no case, however, is the disagreement in regard to these characters so marked as that in respect to color characters.

2. The relative amount of divergence among the observers in regard to the distribution of the kernels in color classes is strikingly different for different ears. Ear No. 9 plainly bore kernels which were relatively easy to classify. The same was true of ear No. 10. On the other hand the kernels of ears 8 and 11 offered many difficulties in classification. But in the case of ear No. 11 the difficulty was largely confined to the sweet kernels, there being close agreement between all the observers but one

Table IV.

Showing the Classification of the Kernels of Ear No. 10 by the Different Observers.

Observer.	Classes of Kernels.					
	Yellow Starchy.	Yellow Sweet.	White Starchy.	White Sweet.	Total Starchy.	Total Sweet.
Mendelian expectation	243.00	81.00	81.00	27.00	324.00	108.00
I.	251	51	85	45	336	96
II.	251	53	85	43	336	96
III.	248	51	88	45	336	96
IV.	260	65	76	31	336	96
V.	245	65	91	31	336	96
VI.	251	75	85	21	336	96
VII.	246	61	90	35	336	96
VIII.	247	75	89	21	336	96
IX.	249	70	87	26	336	96
X.[1]	247	74	89	22	336	96
XI.[1]	250	73	85	23	335	96
XII.[1]	247	71	88	25	335	96
XIII.[1]	247	76	88	20	335	96
XIV.[1]	250	74	85	22	335	96
XV.[1]	246	78	89	18	335	96
Totals.	3,735	1,012	1,300	428	5,035	1,440
Means.	249.00	67.47	86.67	28.53	335.67	96.00

(No. 1) with regard to the yellow and white starchy kernels of this ear.

3. The cause of the discrepancies between the counts of the several observers is obvious from the data. It will be seen at a glance from the diagrams that generally when an observer's count of the *yellow* starchy kernels of an ear, for example, deviated from the mean in excess, this same observer's count of the *white* starchy kernels deviated from the mean in defect, and by an amount approximately corresponding to the positive deviation in the other case. In other words, certain kernels, either starchy or sweet, which were called "yellow" by one observer were called "white" by another. This brings out in a striking way what was obvious to each observer who handled this maize, namely, that there were on each ear a number of both starchy and sweet kernels which were intermediate in respect to color. The distribution of such kernels into the Mendelian categories depends upon the

[1] Between the time when ear No. 10 was counted by observer X. and observer XI. one starchy kernel was lost. Consequently the totals on this ear (sum of all starchy and all sweet kernels) are smaller by one for observers XI. to XV. inclusive than for the other observers.

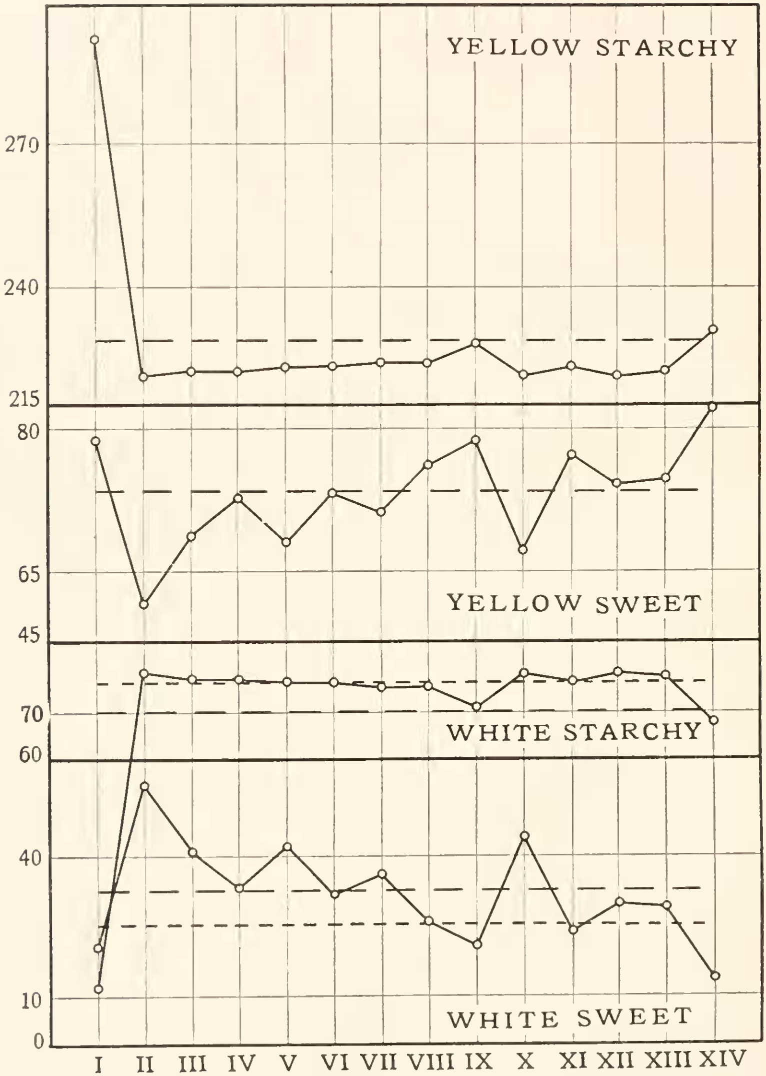

FIG. 4. Diagram showing the count of the different observers of each of the four classes of kernels for ear No. 11.

personal "equation" or bias of each individual observer. As a matter of fact it was possible (and this was done) to make a perfectly graded series of either starchy or sweet kernels from a single ear which ranged from pure white at one end to pure deep

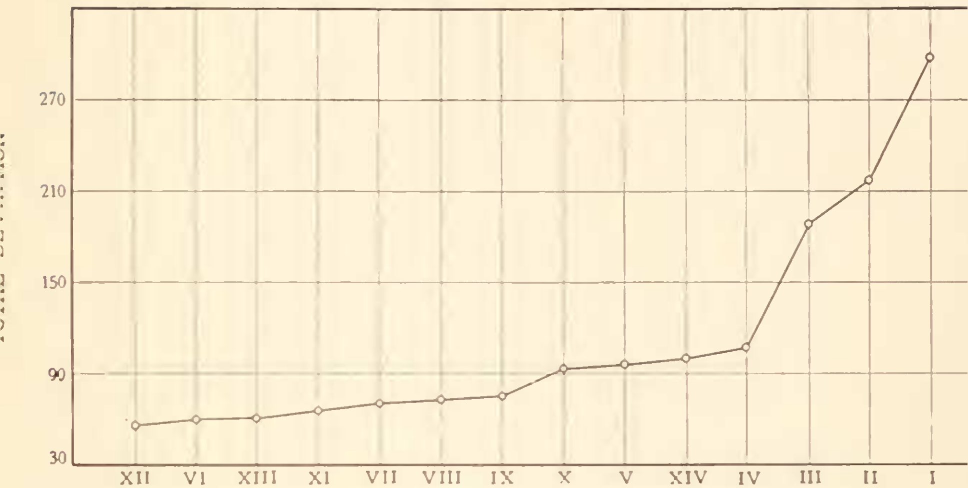

FIG. 5. Diagram showing the total deviations in all the counts of all observers.

TABLE V.

SHOWING THE CLASSIFICATION OF THE KERNELS OF EAR NO. 11 BY THE DIFFERENT OBSERVERS.

Observer.	Classes of Kernels.					
	Yellow Starchy.	Yellow Sweet.	White Starchy.	White Sweet.	Total Starchy.	Total Sweet.
Mendelian Expectation.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
I.	292	87	7	21	299	108
II.	221	53	78	55	299	108
III.	222	67	77	41	299	108
IV.	222	75	77	33	299	108
V.	223	66	76	42	299	108
VI.	223	76	76	32	299	108
VII.	224	72	75	36	299	108
VIII.	224	82	75	26	299	108
IX.	228	87	71	21	299	108
X.	221	64	78	44	299	108
XI.	223	84	76	24	299	108
XII.	221	78	78	30	299	108
XIII.	222	79	77	29	299	108
XIV.[1]	231	94	68	14	299	108
Totals.	3.197	1.064	989	448	4.186	1.512
Means.	228.36	76.00	70.64	32.00	299.00	108.00

[1] No count of this ear by observer No. XV. is included in this table.

yellow at the other end, with each intermediate step practically as small as one cared to make it. An attempt was made to obtain photographs of such series of kernels which would demonstrate the fact of this gradation pictorially, but the photographic resources at command were not equal to the task and it had to be abandoned.

4. The data presented fully demonstrate, I think, the interesting fact that if each of these fifteen competent, and with one exception (No. X.), specially trained observers had independently undertaken an investigation of Mendelian inheritance in maize, and all used the same seed, of at least the two strains here employed, grown their crops in the same place, and even studied *identically the same* progeny ears, *no two would have fully agreed in the numerical values of the* F_2 *ratios.*

II.

Let us now consider the question as to whether these deviations due to personal equation are of sufficient magnitude to be practically significant. The whole of the remainder of this paper will be devoted to a discussion, from different standpoints, of the quantitative aspects of the recorded classifications of the several observers. All these data will bear upon this general point. To answer the question specifically raised in this section it will only be necessary to show the range of the variation exhibited in the counts made. Table VI. gives for the four ears and the four classes of kernels on each ear (*a*) the mean numbers of kernels found by averaging the counts of all observers, (*b*) the minimum and the maximum recorded number of kernels, (*c*) the total range of variation shown in the records, and (*d*) the percentage which this range is of the mean of the same class.

It is evident from this table that the personal element is one of real significance. When two careful observers can differ in their count of the same set of objects by as much as one and a half times the actual number of the objects counted the factor which leads to this difference is certainly not to be neglected.

An examination of the standard deviations and coefficients of variation of the counts leads to the same result. These constants are shown in Table VII. It should be said in this

Table VI.

Showing the Range of Variation Exhibited by All Observers in the Several Classifications.

Ear No.	Class.	Mean.	Lowest Count.	Highest Count.	Range.	Percentage of Range in Mean.
8	Yellow starchy.	316.87	298	352	54	17.0
8	Yellow sweet.	93.47	49	104	55	58.8
8	White starchy.	86.67	52	108	56	64.6
8	White sweet.	35.60	25	79	54	151.7
9	Yellow starchy.	240.93	227	249	22	9.1
9	Yellow sweet.	75.40	62	82	20	26.5
9	White starchy.	81.00	74	93	19	23.4
9	White sweet.	24.67	18	37	19	77.0
10	Yellow starchy.	249.00	245	260	15	6.0
10	Yellow sweet.	67.47	51	78	27	40.0
10	White starchy.	86.67	76	91	15	17.3
10	White sweet.	28.53	18	45	27	94.6
11	Yellow starchy.	228.36	221	292	71	31.1
11	Yellow sweet.	76.00	53	94	41	53.9
11	White starchy.	70.64	7	78	71	100.5
11	White sweet.	32.00	14	55	41	128.1
Mean.						56.2

connection that for the particular sort of problem here dealt with it would appear that the method of expressing the degree of variability which is used in Table VI. (*i. e.*, the absolute value of the range and its relation to the mean) is probably of more real value than are the conventional constants given in Table VII. In the present instance it is the *range* of variation (*i. e.*, the extreme amounts by which different observers differ in their counts) which is the thing of primary interest and practical significance.

Table VII. gives the standard deviation and coefficient of variation for the counts of each class of kernels.

Table VII.

Showing the Absolute and Relative Variation in the Counts of the Several Classes of Kernels.[1]

Ear No.	Yellow Starchy.		White Starchy.		Yellow Sweet.		White Sweet.	
	Standard Deviation.	Coeff. of Var.	Standard Deviation.	Coeff. of Var.	Standard Deviation.	Coeff. of Var.	Standard Deviation.	Coeff. of Var.
8	13.44	4.24	12.90	14.98	13.85	14.82	13.58	38.14
9	6.09	2.53	4.84	5.98	4.91	6.51	4.69	19.00
10	3.52	1.41	3.46	3.99	9.08	13.46	9.08	31.84
11	17.86	7.82	17.86	25.28	10.52	13.84	10.52	32.88

[1] Since these constants are not used in any detailed comparisons it has not been thought necessary to calculate probable errors. All the necessary data are at hand, however, if anyone wishes to make these computations. It need only be remembered that for ears 8, 9 and 10, $n = 15$, and for ear 11, $n = 14$.

This table brings out several points which need discussion. These are:

1. The amount of variation, both absolute and relative, in the counts is shown by the measures here used to be very large for some ears and classes of kernels. For no ear, taken as a whole, can the variation fairly be considered negligible. Thus the conclusion previously reached by another method is confirmed.

2. The amount of variation in the sorting and counting is distinctly different for the different ears. From the values of the constants it would appear that ear No. 11 presented the greatest difficulty in respect to the classification of starchy kernels. In respect to sweet kernels ears No. 8 takes rank as offering the greatest difficulties. The starchy kernels of ear No. 10 were the easiest to classify of all starchy kernels. In the case of sweet kernels ear No. 9 had fewer intermediates (*i. e.*, was easier to classify) than any other ear.

3. *Relatively* there was closest agreement among the observers in respect to yellow starchy kernels, and least agreement in respect to white sweet kernels. This table illustrates the fact which was evident to the observers themselves, that there were marked differences in the ease with which the kernels of different ears and different classes could be sorted.

Now while it has been shown that the fifteen observers do not agree in their classification and counts, and that the differences are too large to be neglected, it may fairly be asked if the same result would appear if the group of observers participating were not merely scientifically trained and familiar with maize, but in addition had had a considerable amount of actual experience in the detailed study of variation and inheritance in plants. In other words, is not that special familiarity with the object which comes with the active prosecution of research in a particular field worth something in reducing the magnitude of one's personal error or "equation"? To get some light on this point Table VIII. has been prepared. This is made up in exactly the same way as Table VI., *except* that only observers VI., VII., VIII., IX., XI., XII., XIII. and XIV. are included. These eight observers, comprising the staffs of Professor Johannsen's and the writer's laboratories, have certainly had more extended experience in the direct and immediate study of plant breeding and of variation in plants (involved in all breeding investigation) than have the other observers of the original fifteen. Lists of published papers could be cited in proof of this were it necessary, but the fact is obvious. Will this group of workers on problems of variation and inheritance show a similar degree of variability in their counts to that brought out in Table VI.?

Table VIII.

Showing the Range of Variation Exhibited by Observers VI. to IX. Inclusive, and XI. to XIV. Inclusive.

Ear No.	Class.	Mean.	Lowest Count.	Highest Count.	Range.	Percentage of Range in Mean.
8	Yellow starchy.	312.88	308	328	20	6.4
8	Yellow sweet.	98.13	86	104	18	18.3
8	White starchy.	90.50	78	95	17	18.8
8	White sweet.	30.50	25	43	18	59.0
9	Yellow starchy.	242.88	240	246	6	2.4
9	Yellow sweet.	77.00	75	80	5	6.5
9	White starchy.	79.75	77	82	5	6.3
9	White sweet.	22.38	21	25	4	17.9
10	Yellow starchy.	248.38	246	251	5	2.0
10	Yellow sweet.	71.88	61	76	15	20.9
10	White starchy.	87.13	85	90	5	5.7
10	White sweet.	24.13	20	35	15	62.2
11	Yellow starchy.	224.50	221	228	7	3.1
11	Yellow sweet.	81.50	72	87	15	18.4
11	White starchy.	74.50	71	78	7	9.4
11	White sweet.	26.50	21	36	15	56.6
Mean						19.6

It is seen from comparison of this table with Table VI. that the amount of variation in the sorting and counting is distinctly reduced in the group of students of variation. Whereas the average percentage of range in mean is 56.2 for Table VI., it is but 19.6, or only approximately *one third* as much, for Table VIII. Thus it appears that in this case, just as would be expected on general grounds, special experience or practice in a particular line greatly reduces the personal equation. It must be said, however, that even with the group of observers included in Table VIII., the differences are too large to be neglected. When the range of variation amongst different observers of the same thing amounts on the average to approximately one fifth (19.6 per cent.) of the mean value of the thing counted it indicates a source of error not lightly to be dismissed.

III.

It is desirable next to examine somewhat more closely into the nature and distribution of the discrepancies among the observers. A point of particular interest is to determine to what extent the counts indicate a definite and persistent bias on the part of an observer. There may be great variation in the counts of several observers of the same set of things and yet each observer's judgments may be distributed quite at random about the mean. In order to get more light on this and some other matters Table IX. has been prepared. This table gives in successive columns for the four kernel classes, first, the mean deviation from the mean, all deviations being taken together without reference to sign (*i. e.*, the mean total deviation), and second, the mean net deviation from the mean, got by taking the algebraic sum of the deviations. All four ears are used in getting these mean deviations. An example will make clear the method of obtaining the values given in this table. An examination of Tables II. to V. inclusive shows the following set of deviations from the means in the counts of yellow sweet kernels by observer No. V.

+ Deviations from Mean.	— Deviations from Mean.
7.53 (ear 8)	2.47 (ear 10)
5.60 (ear 9)	10.00 (ear 11)
13.13 = sum of + deviations	12.47 = sum of — deviations.

$$\frac{13.13 + 12.47}{4} = 6.40 = \text{mean } \textit{total} \text{ deviation from mean.}$$

$$\frac{13.13 - 12.47}{4} = +0.165 = \text{mean } \textit{net} \text{ deviation from mean.}$$

The last column of the table gives the total deviation from the mean of each observer, all ears being taken together and the deviations summed without regard to sign.

It is strikingly evident from the mean net deviations in this table that each observer was "a law unto himself." Nearly every one of the fifteen evidently had a different system of sorting.

Table IX.

Showing the Mean Deviation from the Mean (Total and Net) and Total Deviations of the Counts of all Observers.

Observer.	Yellow Starchy.		Yellow Sweet.		White Starchy.		White Sweet.		Total Deviation on All Ears and All Classes.
	Mean Total Deviation from Mean.	Mean Net Deviation from Mean.	Mean Total Deviation from Mean.	Mean Net Deviation from Mean.	Mean Total Deviation from Mean.	Mean Net Deviation from Mean.	Mean Total Deviation from Mean.	Mean Net Deviation from Mean.	
I.	26.93	+23.46	10.10	− 0.34	26.995	−22.995	10.44	+ 0.14	297.82
II.	6.04	+ 2.36	22.09	−22.09	4.68	+ 1.84	21.55	+21.55	217.40
III.	9.29	− 9.29	14.34	−14.34	10.26	−10.26	13.30	+13.30	188.72
IV.	11.04	+ 7.86	2.86	− 0.93	9.93	− 6.75	2.94	− 1.20	107.04
V.	8.79	− 8.79	6.40	+ 0.17	3.34	+ 3.00	5.55	+ 5.55	96.32
VI.	3.54	− 1.08	4.42	+ 4.42	2.59	+ 1.76	4.70	− 4.70	60.98
VII.	4.04	− 4.04	4.59	− 4.59	4.26	+ 4.26	4.55	+ 4.55	70.72
VIII.	3.08	− 3.04	5.92	− 5.92	3.26	+ 3.26	6.20	− 6.20	73.78
IX.	2.89	+ 2.71	6.42	+ 6.42	2.84	− 2.49	6.70	− 6.70	75.38
X.	4.83	− 4.29	6.65	− 0.085	5.26	+ 5.26	6.65	− 0.05	93.52
XI.	4.08	− 1.54	4.37	+ 4.17	3.84	+ 1.51	4.45	− 4.45	66.92
XII.	3.83	− 2.79	3.17	+ 3.17	3.51	+ 3.01	3.70	− 3.70	56.87
XIII.	5.08	+ 0.77	1.67	+ 1.67	4.51	+ 3.51	3.95	− 3.95	61.58
XIV	3.40	+ 0.90	9.17	+ 9.17	3.16	− 0.25	9.45	− 9.45	100.68
XV.[1]	8.07	+ 6.07	4.82	+ 4.55	6.67	− 5.11	5.93	− 5.93	76.46

[1] On three ears only.

Some of these differences are very interesting. For example: No. I. had a high net deviation on starchy kernels, tending to overestimate the yellows and practically zero net deviation on sweet kernels; XIV. is exactly the opposite, having very small net deviations on the starchy and tending strongly to overestimate the yellows among the sweet kernels. No. II. had the tendency to underestimate the yellow sweets, and correspondingly to overestimate the white sweets. No. III. consistently underestimated rather heavily all yellows and overestimated all whites. No. IV. did precisely the opposite. No. V. shows a very erratic set of net distributions, owing to his idiosyncrasy respecting the discrimination between starchiness and non-starchiness. The result is that while he underestimated the yellow starchy kernels, he overestimated all the other classes. No. VI. somewhat underestimated the yellow starchy, but overestimated the yellow sweet. No. X. shows an extraordinarily small net deviation on the sweet kernels, but distinctly underestimated the yellow starchy. In general the table shows in a striking way, that the individuality of the observer is a factor to be reckoned with in work of this sort.

It is of some interest to examine the trend of the total deviations given in the last column. The data are shown graphically in Fig. 5, arranged in order from the smallest to the greatest deviation.

This diagram illustrates a point frequently overlooked. It is commonly argued that the more independent judgments one obtains regarding any point the more accurate will the average result be. We are apt to say that if ten men measure a stick the average of their measurements will necessarily be nearer to the true dimension than if but three men measure and their average be taken. But it is plainly evident from Fig. 5 and Tables VI. and VIII. that the inclusion of observers I., II., III. added nothing to the accuracy of the mean. The point which is forgotten in assuming that greater numbers necessarily mean greater accuracy is apparent if we examine the equation for the probable error of a mean which is

$$P.E._M = .67449 \frac{\sigma}{\sqrt{n}}.$$

The probable error, to be sure, varies inversely with n, but it also varies directly with σ, the standard deviation. And, what is here of primary importance, the standard deviation tends to increase as n increases. Whether the probable error shall be smaller or not as the number of observations is increased depends upon what has happened in the meantime to the standard deviation. When n is small, as in the case here under discussion, the effect on the standard deviation of taking $n + 1$ observations as compared with n may greatly outweigh its effect in the denominator of the probable error fraction.

IV.

The next point to be considered is the relative constancy of the same observer's error. If each of the fifteen observers had made a second count of all the ears at some considerable interval of time after the first, how closely would the recounts tally with the original counts? Such an experiment really tests, of course, the stability or constancy of an observer's judgment. It indicates the degree to which his standard of sorting is absolute, and to what extent it fluctuates.

It was not feasible to ask all of the original fifteen observers to go to the labor of recounting these ears. Second counts made after a relatively long lapse of time are, however, available from three observers (namely, VI., VIII. and IX.) for all four ears. While this gives only comparatively meager data, still some points of interest appear. These data are given in Tables X., XI. and XII. It should be said that the recounting was done in the same way as the original count. In each case the observer had no access to the original data while the second count was in progress. No one of the three had any remembrance of what his (or her) original counts were. The writer has not been able to discover any factor which would make these recounts anything other than what they were intended to be, namely, really independent determinations of the same material by the same observers after a long lapse of time.

It will be remembered (*cf.* p. 349 *supra*) that one kernel from ear No. 10 was lost in the course of the original counting. It is therefore obvious that all the recounts of this ear must of necessity be one kernel smaller than the first counts.

TABLE X.

ORIGINAL AND SECOND COUNTS OF EARS 8 TO 11 BY OBSERVER NO. VI.

Ear and Count.	Classes of Kernels.				Date.
	Yellow Starchy.	Yellow Sweet.	White Starchy.	White Sweet.	
8, Original.	313	100	90	29	January 21, 1910.
8, Recount	312	100	91	29	August 10, 1911.
Difference.	−1	0	+1	0	
9, Original.	242	79	81	20	January 21, 1910.
9, Recount.	240	76	83	23	August 11, 1911.
Difference.	−2	−3	+2	+3	
10, Original.	251	75	85	21	January 21, 1910.
10, Recount.	244	73	91	23	August 11, 1911.
Difference.	−7	−2	+6	+2	
11, Original.	223	76	76	32	January 21, 1910.
11, Recount.	223	75	76	33	August 10, 1911.
Difference.	0	−1	0	+1	

The data in these tables indicate that, so far at least as these three observers are concerned, the judgment of the individual is reasonably constant. This is plain if the total deviation of

TABLE XI.

ORIGINAL AND SECOND COUNTS OF EARS 8 TO 11 BY OBSERVER NO. VIII.

Ear and Count.	Classes of Kernels.				Date.
	Yellow Starchy.	Yellow Sweet.	White Starchy.	White Sweet.	
8, Original.	311	101	92	28	January 20,1910.
8, Recount.	309	93	94	36	August 12, 1911.
Difference.	−2	−8	+2	+8	
9, Original.	241	78	82	21	January 20, 1910.
9, Recount.	243	79	80	20	August 12, 1911.
Difference.	+2	+1	−2	−1	
10, Original.	247	75	89	21	January 20, 1910.
10, Recount.	246	73	89	23	August 12, 1911.
Difference.	−1	−2	0	+2	
11, Original.	224	82	75	26	January 20, 1910.
11, Recount.	223	81	76	27	August 12, 1911.
Difference.	−1	−1	+1	+1	

TABLE XII.

ORIGINAL AND SECOND COUNTS OF EARS 8 TO 11 BY OBSERVER NO. IX.

Ear and Count.	Classes of Kernels.				Date.
	Yellow Starchy.	Yellow Sweet.	White Starchy.	White Sweet.	
8, Original.	327	101	78	26	January 21, 1910.
8, Recount.	314	98	89	31	August 11, 1911.
Difference.	−13	−3	+11	+5	
9, Original.	242	80	79	21	January 21, 1910.
9, Recount.	240	76	83	23	August 11, 1911.
Difference.	−2	−4	+4	+2	
10, Original.	249	70	87	26	January 21, 1910.
10, Recount.	246	71	89	25	August 11, 1911.
Difference.	−3	+1	+2	−1	
11, Original.	228	87	71	21	January 21, 1910.
11, Recount.	222	88	77	20	August 12, 1911.
Difference.	−6	+1	+6	−1	

recounts from original counts be considered. In the case of observer No. VI. a total of sixteen kernels were differently classified in the recount from what they were originally. Since the whole number of kernels involved in the experiment was 1,792, this means a discrepancy of less than one per cent. between two independent sortings more than a year apart. Such an error is certainly negligible. Observer No. VIII. classified eighteen kernels, all told, differently in the recount than in the original. This again is only about one per cent. of the total kernels handled, and cannot be regarded as a significant error. In both of these cases (VI. and VIII.) the discrepancies had to do entirely with the color classification. With observer IX. this was not the case. On both ear 8 and ear 9 she classed two kernels as sweet in the recount which she had originally called starchy. Altogether this observer classified thirty-five kernels differently in the recount from what she did in the original. This however represents a relative error of a little less than two per cent. No very great stress could be laid upon such an error.

From the tables it will be noted that there was a marked and nearly uniform tendency on the part of all three observers to underestimate the yellows (both starchy and sweet) and to overestimate the whites, in the recounts as compared with the originals. It seems probable that the cause of this lies, in part at least, in a fading of the yellow color during the time since the first counts were made. Thus it may be that kernels which were plainly yellow when first counted are now white or very nearly so. A further fact which would indicate that fading had occurred is found in the mental impressions of the observers. All three found the material distinctly more difficult to classify when recounted than when originally counted. One feels certain that a part, at least, of this is due to a change in the material itself.

Recognizing fully the meagerness of the material, the facts so far as they go seem to indicate clearly that the same observer is likely to classify the same material in about the same way every time. If a particular kind of bias is shown in one count it will appear essentially unaltered in successive trials. This is probably more true of observers especially experienced in dealing with the data of variation than in the case of those without such experience, though figures are lacking to demonstrate this.

V.

We come next to the consideration of the second question proposed at the beginning (p. 341). This was: "Does somatic 'intermediateness' in maize imply gametic 'intermediateness'? In other words, do F_2 kernels which are intermediate *somatically* give rise to any different sort of progeny when planted than do kernels which belong clearly and indubitably to one or another of the well defined gametic classes in F_2?" To answer this question carefully controlled plantings of somatically intermediate kernels were made in 1910. Series of starchy and of sweet kernels were formed ranging in each case from pure white at one end to pure deep yellow at the other end. Then rows were planted as follows: (1) pure white, (2) deep yellow, (3) the lightest yellow to be found (= somatic intermediates), (4) the yellowest whites to be found (= somatic intermediates). The kernels in classes (3) and (4) were such as would be classified with the yellows by some observers and with the whites by others. The rows included about twenty plants each and were made in duplicate, and in some instances triplicate for both starchy and sweet series. In each row a varying number of ears were self-fertilized (*i. e.*, pollinated by hand with pollen borne on the same plant). Owing to the numerous vicissitudes incident to hand-pollination, together with pressure of other work, as large a number of good ears as would be desirable was not obtained. Some of the possible gametic combinations were not represented at all in the progeny ears. This part of the investigation is, in consequence, not complete. It seems desirable, however, to present briefly the general result shown by the fifty odd ears at hand.

This result was that there was no discernible difference whatever between the progeny of groups (1) and (2) *as a class*, and that of groups (3) and (4) *as a class*. In (3) and (4) some of the kernels planted were of course heterozygotes and some were homozygotes. The same was true, however, of the kernels of (1) and (2). In each case a typical Mendelian result was obtained, and this result could have been predicted in every case (with the exception to be noted presently) had the *gametic* constitution of the kernel been known when it was planted. It could not have been predicted from the *somatic* appearance of the kernel.

The only behavior of an exceptional character observed in these selfed ears was that in certain of the white sweet kernels, which were homozygous recessives in respect to absence of yellowness and starchiness, selfing brought out a latent red.[1] The three ears of this type which were obtained all came from kernels classified in the planting as pure white (group (1)). No such ears were obtained from selfed sweet kernels in group (4). The total number of homozygous, non-yellow sweet ears obtained was too small, however, to make it at all certain that similar red ears might not, with larger numbers, be obtained from group (4) kernels.

It is planned to get further data on this portion of the investigation, using for planting the kernels of ears 8, 9, 10 and 11 which formed the material for the personal equation part of the work. It can be said at this time that the experiments with cross-bred maize so far conducted furnish no evidence that somatic "intermediateness" connotes gametic intermediateness. The progeny of a deep yellow kernel selfed is not visibly different from that of a light yellow kernel selfed, provided both are of the same gametic constitution. The result of this experiment precisely agrees with Darbishire's[2] extensive study of essentially the same problem with peas. Indeed his final conclusion (*loc. cit.*, p. 71) applies here without change of wording: "That in the attempt to predict the result of a given mating the somatic character not only of the parents and of the ancestors of the individuals mated, but of the individuals themselves, may be entirely left out of account; and that the expectation based on a theory of the contents of the germ cells of the two individuals is fulfilled

Discussion and Summary.

Results such as are set forth in this paper would certainly have been at one time proclaimed by some as furnishing a refutation of Mendelism. In fact one of the earliest criticisms[3] of Mendelian work was mainly devoted to calling attention to the existence of such somatic intermediates between Mendelian categories in the

[1] A similar result has recently been described by Emerson, R. A. Rept. Amer. Breeders' Assoc., VI., 233–237, 1911.

[2] Darbishire, A. D., "An Experimental Estimation of the Theory of Ancestral Contributions in Heredity." *Proc. Roy. Soc.*, B, Vol. 81, pp. 61–79, 1909.

[3] Weldon, W. F. R., "Mendel's Laws of Alternative Inheritance in Peas." *Biometrika*, Vol. I., pp. 228–254, Plates I. and II., 1902.

case of peas as are here shown to exist in maize. That such variation, provided it be really somatic or fluctuational, is, however, of no real importance in relation to the cardinal facts of Mendelian inheritance has been shown by all experimentalists who have devoted attention to the matter. Bateson[1] (*loc. cit.*, pp. 240–244) gives an illuminating discussion of the whole matter, with special reference to the phenomena in peas. East and Hayes[2] discuss the same point with reference to maize and show that somatic intermediates behave in inheritance in accord with their gametic constitution rather than their somatic appearance. Certainly the time is past when facts such as are set forth in the present paper can be adduced in criticism of basic Mendelian principles.

The essential point brought out by this study is, it seems to me, that the well known *general* fact that every datum of science is a function (in the mathematical sense) of two variables, namely, the observer and the thing observed, is once more emphasized by a particular case.

A thorough investigation which brings out essentially this same point, though conducted on a different class of material and with a somewhat different object in view, has been made by Yule.[3]

It will be freely admitted by everyone as an abstract proposition that the personal idiosyncracy of the observer constitutes a source of error in all scientific observing. Yet how often does the biologist not working on strictly quantitative problems make any effort either to eliminate or determine the magnitude of this source of error in his case and in a specific instance? Anyone who has not experimented for himself on the matter can hardly realize how important, on the one hand, and how difficult on the other hand, it is to attain to any considerable degree of real objectivity in results. While the "exact" sciences are somewhat better off in this regard than biology, they are after all not greatly so. There has, to be sure, been a great deal of work done

[1] Bateson, W., "Mendel's Principles of Heredity." 2d Edit., Cambridge, 1909.

[2] East, E. M., and Hayes, H. K., "Inheritance in Maize." Conn. Agr. Expt. Stat., Bulletin 167, 1911.

[3] Yule, G. U., "On the Influence of Bias and Personal Equation in Statistics of Ill-defined Qualities." *Jour. Anthropol. Inst.*, Vol. XXXVI., pp. 325–381, 1906.

on the theory of errors of observation, particularly as related to astronomy, physics, and like subjects, yet so late as 1902 Pearson[1] demonstrated in a most convincing manner that much of the then currently accepted theory was wrong, and that all of it quite overlooked a factor which might be exceedingly important, namely, correlation of judgments.

The present study is by no means a complete investigation of the problem of personal equation in Mendelian work. Correlation of errors ought to be studied, and certain other matters as well. But the present material is statistically entirely inadequate for the discussion of these points, and it does not seem feasible to collect more extensive data, since to do so involves too great a trespass on the time and good nature of busy workers. Further the material here presented brings out clearly the primarily essential points. It shows that in a Mendelian ratio the personal equation of the observer marks a source of error which in the case of maize is of considerable magnitude. This source of error quite overshadows in magnitude, in this case, the error due to random sampling. Yet it is the latter alone which is ordinarily considered by Mendelian workers. The probable error of a Mendelian ratio as commonly calculated tells one the probability that the sample counted is a true representation of the general population from which it was drawn. It tells one nothing whatever about the unconscious bias of the counter as a factor in producing the result set down.

By way of summary it may be said that in this paper evidence is presented which shows that:

1. The observed F_2 Mendelian ratios determined from the same four ears of maize by fifteen competent observers all differ from one another.

2. The failure of all observers to agree in their distribution of kernels into several categories results from two causes, viz., (*a*) the existence of somatically intermediate kernels, and (*b*) the personal bias or idiosyncracy of the observer.

3. The magnitude of the differences between the several observers is such as to demonstrate that the personal equation

[1] Pearson K., "On the Mathematical Theory of Errors of Judgment, with Special Reference to the Personal Equation." *Phil. Trans. Roy. Soc.*, A, Vol. 198, pp. 235–299, 1902.

is a factor which cannot safely be neglected in work of this character.

4. The observers who have had most experience in the appreciation and measurement of variation have the smallest personal equations on the class of material and the problem here treated.

5. There is no evidence that the progeny of somatically intermediate kernels is different, in any respect whatsoever, from the progeny of distinctly non-intermediate kernels of the same gametic constitution.

DIFFERENTIATION OF THE HUMAN CELLS OF SERTOLI.

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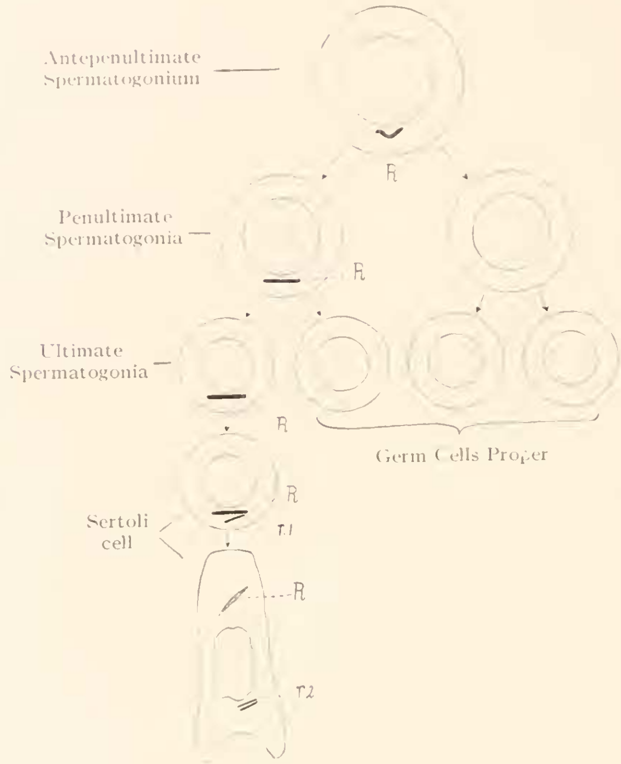
This study is based on the examination of the testis of a negro about 40 years of age, preserved in Zenker's fluid while still warm after his execution. The fixation was not as excellent as might be desired, cytoplasmic details being not always preserved, but the preservation of the nuclei was on the whole very good, and of spindle figures excellent. A considerable variety of staining methods were employed, of which the most fruitful proved to be Heidenhain's iron hæmatoxyline, with various degrees of extraction, followed by alcoholic eosin. Paraffine sections were made of 5μ and 8μ.

For the gift of this material I am indebted to the kindness of Dr. Addison, of the University of Pennsylvania.

1. General Outline of the Process.

The text diagram exhibits the chief results obtained. The antepenultimate spermatogonia contain each a rod (*R.*) within the cytoplasm. This does not divide in mitosis, consequently just half of their daughter cells, the penultimate spermatogonia, come to contain each a rod, while half of them lack it. In the division of these penultimate spermatogonia the rod does not divide but becomes distributed to one quarter of the ultimate spermatogonia. Each of every three ultimate spermatogonia produces by division two primary spermatocytes, and these cells which belong to the true germinal cycle lack the rod entirely. But each fourth ultimate spermatogonium preserves the rod, and this cell without further division enlarges and becomes a cell of Sertoli. In this cell of Sertoli a primary rodlet (*r. 1*) buds off from the rod; then the rod disappears, while the primary rodlet divides into two secondary rodlets (*r. 2*) and the latter persist in the Sertoli cell throughout its history.

The line of the Sertoli cell is therefore determined by the presence of the rod; one Sertoli cell is produced to every three



ultimate spermatogonia that lack the rod, or one Sertoli cell to every twenty-five spermatids.

2. The Antepenultimate Spermatogonia (Figs. 1–8, Pl. I.).

These are the largest germ cells in the adult testis, and like the other generations of spermatogonia are situated at the periphery of the seminiferous tubules. Frequently their nuclei are of irregular shape, as shown in Fig. 1. Within the nuclei (Fig. 3) are two kinds of nucleolar structures: acidophilic plasmosomes and basophilic bodies; it would take a detailed study to determine whether the latter are chromatoid nucleoli or modified chromosomes (allosomes). In their cell bodies are found chromatic rods, never more than one to a cell, various forms of which are drawn in Figs. 2–8, that of Fig. 5 being the largest found. The rods are homogeneous in appearance, dense, and they stain with basic stains but usually not as intensely as in the later spermatogonial generations. Such rods are usually in contact with the nuclear surface, but not always, and do not occupy constant positions with regard to the poles of the cell. Characteristic of the antepenultimate spermatogonia is the relatively small size of these rods and their frequently twisted form.

Thirty of these cells were carefully examined, and twenty-three of them showed each one rod. Of the remaining seven, five were not wholly within the plane of the section, so that their rods may have been present in the excised portions. It is probable that each cell of this generation comes to contain a rod, and that the rods are first produced in this generation; no spermatogonia of earlier generations were present, however, consequently there can be no surety of the latter point.

What the method of origin of these rods may be could not be determined. They are quite distinct from the idiozome, at least when fully formed, as shown in Fig. 2. In one case (Fig. 1) no rod was found but an irregular granular mass (*x*) which is possibly a precursor of the rod; if this be so, the rod might be considered to be produced by the conglomeration of granules at first scattered in the cell body. But the state of fixation of the cytoplasm was not sufficiently reliable to allow of any satisfactory determination of this matter. There is no evidence that the rods are directly produced from the nucleus. Their origin is thus unexplained, though their subsequent history is perfectly clear.

No mitoses of these cells were found, but there can be no doubt that the rods do not become divided for just half of the cells of the next generation contain rods.

3. The Penultimate Spermatogonia (Figs. 9–16).

Forty-nine cells of this generation were examined, care being taken to study only those that lay entirely within the plane of the section, and of these twenty-four exhibited each a rod while twenty-five showed no rods. Also six cases were found of two nuclei in one cell body, indicating nuclear division without cytoplasmic division of antepenultimate spermatogonia: in all six of these cases only one rod was present, and an example is shown in Fig. 9. There can thus be no doubt that half the penultimate spermatogonia contain rods and half do not.

Characteristic appearances of the rods are illustrated in Figs. 9–11, Pl. I., 14–16, Pl. II. They differ on the average from those of the preceding generation in being usually larger, straighter, and more deeply-staining with hæmatoxyline, which indicates they have been undergoing growth changes.[1] Not infrequently they are curved around the nuclear surface (Fig. 14) and the length of a rod may equal the diameter of a nucleus. Consequently they are in this stage very prominent constituents of the cell bodies and easily differentiated by safranine or hæmatoxyline.

Mitoses of these cells were not frequent, but two clear cases (Pl. II., Figs. 12, 13) were found, showing that the rod (*R.*) passes undivided into one of the daughter cells, and this is fully borne out by a study of their distribution in cells of the following generation. Fig. 18 shows the end result of such a mitosis in a case where the cell body had not divided and here there is but one rod. What is the nature of the scattered globules shown in Figs. 12 and 13 is doubtful; they may be discharged nucleolar material.

4. The Ultimate Spermatogonia (Figs. 17–24, Pl. II.).

These are the smallest of the spermatogonia and the most numerous in the testis studied. One quarter of them contain each a rod; three quarters lack rods. One hundred and forty-two of these cells were studied, at stages before any of them had enlarged into Sertoli cells, the precaution being taken to include only cells lying wholly within the section; of these twenty-five showed each one rod, and one hundred and seventeen showed no rods. This ratio is somewhat less than 1 : 3, which is readily explained on the ground that some of the spermatogonia with rods had already become Sertoli cells and therefore were not included in the count. A very important and clear case is that

[1] The condition of the pair of rodlets (*r. 2*) in Fig. 10 will be explained later.

of Fig. 17; this shows four nuclei, the granddaughters of the nucleus of an antepenultimate spermatogonium, while there has been no division of the cell body, and it will be seen there is but one rod to the four nuclei. The evidence is then decisive that one quarter of the cells for this generation contain each one rod.[1] In these cells the rods are on the average more massive than in preceding generations (Figs. 17–24), and while usually more or less curved are never twisted. Quite frequently one end of the rod is bent off at an angle (Figs. 17, 22). In these cells also the rods are most dense and acquire their maximum stain, staining fully as intensely as the basichromatin; they generally but not always touch the nuclear surface.

5. Differentiation and History of the Sertoli Cells (Figs. 25–50).

All the ultimate spermatogonia that contain rods become cells of Sertoli, and those only. Nothing like either rods or rodlets were found in any of the spermatocytes or spermatids. The Sertoli cells become especially marked by their great growth. Fig. 25, Pl. II., shows the beginning of such growth, the Sertoli cell (*A*) growing out beyond its sister ultimate spermatogonia (*B* and *C*). Figs. 34 and 35, Pl. III., show Sertoli cells in a later growth stage in their entirety, and Figs. 36–39, 41–46 exhibit portions of them in still later stages. These cells became relatively enormous as shown in Fig. 50, Pl. V., which represents a portion of a transection of the wall of a seminiferous tubule; in this figure the shaded portion represents the bodies of the Sertoli cells, which have grown far into the lumen of the tubule to embrace the spermatids. This great growth is due mainly to the formation of vacuoles within the cytoplasm, and in the figures only the larger of the vacuoles are shown, not the great number of minute ones. These vacuoles are drops of a non-staining fluid, like that contained within the cavity of the tubule; only in rare instances are any concretions found in the vacuoles. One end, the basal, of each Sertoli cell remains adherent to the fibrous wall of the tubule and in the figures lines are drawn to denote

[1] Spermatogonia with two, three or four nuclei in a single cell body are unusually frequent and in such cases the sister nuclei are frequently of quite unequal volumes.

the inner border of the tubule; the other end, the distal, is the one that grows out and forms branches ramifying around the spermatocytes and spermatids. In the later history of the Sertoli cells large spaces are found within them, as shown in Fig. 50, which are cavities in which germ cells had been situated before their transformation into spermatozoa. Boundaries between the Sertoli cells become indistinguishable, so that these cells come to constitute a syncytial cytoplasmic net of extremely vacuolar structure (Fig. 50). In the basal portions of the Sertoli cells parallel bundles of fibrils may be seen at certain stages (Fig. 43).

The nuclear changes are also characteristic, and represent a gradual transformation of the structure of the resting nucleus of an ultimate spermatogonium. The reticulum changes first into microsomal masses (Fig. 25, Pl. II.). Then takes place a flowing of these masses together (Figs. 34–39, 41–43, Pl. III., IV.) until all the basichromatic substance of the nucleus becomes concentrated into a mass or karyosphere, and the particles remaining without the mass are oxychromatic. Figs. 44 and 45, Pl. V., represent the result of this process. Then follow stages of dissolution of the karyosphere into minute granules, all of which become gradually oxyphilic (Figs. 46 and 48), Fig. 49 representing a degenerate nucleus at the close of the cell's cycle. During all these stages the nuclei become very irregular, with deep indentations and lobations at their margins and grooves passing along their lengths. This irregularity of form and the central karyosphere are diagnostics by which these nuclei may be readily distinguished from those of neighboring germ cells. Further, the nuclei do not remain at the basal end of the cell, as they do in certain other mammals, but move out beyond the level of the spermatogonia (Fig. 50).

After passing through the series of changes just described the Sertoli cells degenerate, for there is no evidence that they go through a second cycle. This is proven by the later stages of these nuclei (Figs. 47–49) which gradually become wholly achromatic and then disappear from view. Their vacuolar substance must at that time mingle with the fluid of the tubule. Fig. 25 (Pl. II.) is interesting in this regard, for it exhibits a young Sertoli cell (*A*) pushing out before it an old and degenerate one (*D*).

A Sertoli cell is therefore produced to every twenty-four spermatids, and after the latter have metamorphosed into spermatozoa and these spermatozoa have become discharged from the tubules, that Sertoli cell degenerates. Formation of Sertoli cells must then continue through life as long as formation of germ cells continues.

We now pass to the history of the rod in the Sertoli cells, that remarkable body which differentiates them from the functional germ cells. This is at first a simple rod, and may remain such even in the beginning enlargement of the cell (Fig. 25, Pl. II.). But this rod divides, and in most cases before the Sertoli cell begins its growth. Stages of its division are rarely found, and the only ones observed are illustrated in Figs. 26–30, Pl. III. It will be seen that in the cases of Figs. 26–28 the rod is undergoing an unequal longitudinal cleavage, a more slender and shorter rod abstricting from a portion of the larger one; this smaller rod may be called the primary rodlet. Perhaps one reason why these stages are so seldom found is because this division can be seen clearly only when the rod lies at a particular angle of vision. Whether Fig. 30 represents simply an unusually bent rod, or one that is in process of division, is hard to determine, for it was an isolated case. The condition immediately following this division is shown in Fig. 31, with an unusually long primary rodlet (*r. 1*) completely separated from the rod (*R.*); this is a cell body of the volume of that of an antepenultimate spermatogonium, where accordingly cytoplasmic division had not occurred, and where the original nucleus had divided while only one of its daughter nuclei had divided again. A case of rod and primary rodlet together at an unusually late stage is represented in Fig. 41, Pl. IV.

In four fifths of the cases, in 81 out of 100 cells examined, the large rod completely disappears before the Sertoli cell starts in its growth and in such cases only the primary rodlet is to be seen (Figs. 32–33, Pl. III.). Just so soon as the cell enlarges a pair of secondary rodlets are seen instead of the primary rodlet (Fig. 34), and without doubt these are produced by equal longitudinal cleavage of the primary rodlet, for they are always of the same length and lie close together. In their later stages (Figs. 43, 46, 47) these secondary rodlets undergo some increase in thickening, and in all cases these rodlets persist within the Sertoli cell until the end of its cycle; they also probably degenerate there, for no signs of them were found within the germ cells or free in the fluid of the seminiferous tubule.

But in one fifth of the cases, 19 out of 100 cells examined, the original rod continues visible for a shorter or longer period after the secondary rodlets have been produced, as shown in Figs. 35–39, 42, 45; and in Fig. 41 is drawn an unusual case of late persistence of the rod and primary rodlet together. The rod may persist for a while as a single dense body (Fig. 38). Fig. 39 shows a case of such a single rod that has segregated into chromatic and achromatic parts, a rare condition. But as a rule it divides longitudinally as exhibited in Figs. 35–37, 41, 42, 45; this division begins and is most prominent near the middle region of the rod, when its ends may be still undivided, but cases were found where the rod had completely divided into two secondary rods (Figs. 35, 40, Fig. 40 being a rod from a cell of about the stage of the cell shown in Fig. 39). In the instances where the rod persists after the secondary rodlets have been produced, it never stains quite as deeply as the latter, and gradually becomes less and less chromatic until it can no longer be seen; no rod was observed in any cell after the karyosphere of the nucleus had disintegrated.

There is accordingly considerable individual variation in the behavior of the rod after it has abstricted the primary rodlet; in four fifths of the cells it then promptly disappears, in one fifth it persists for a variable period, but never until the end of the cycle of the Sertoli cell, and then undergoes a second longitudinal division which is this time an equal division. The rod when it persists generally remains in the basal portion of the cell body. The secondary rodlets are at first usually in contact with the surface of the nucleus, either basal or distal, while they are later found near the distal pole of the nucleus and usually separated from it. Whether the disappearing rod contributes substance to the formation of the fibers in the cytoplasm (Fig. 43) could not be determined. It is also difficult to decide whether the rods and secondary rodlets are or are not always enclosed in vacuoles.

Certain aberrant cases need mention. In a single instance a pair of secondary rodlets were found together with a rod in a penultimate spermatogonium (Fig. 10), a precocious case of rodlet formation; what the constricted acidophilic body in the cytoplasm of this cell may be, I do not know. Then in each of two cases of rather late Sertoli cells, instead of the general case of one pair of secondary rodlets, two pairs were found (Figs. 44, 48); these might have been produced from an unusually long primary rodlet, such as the one shown in Fig. 31, by the occurrence of a transverse as well as a longitudinal division.

6. Discussion and Conclusion.

It is truly surprising that no thorough account has yet been given of the human cells of Sertoli; indeed, the studies made so far are rather histological than cytological.

Attention to these cells was first drawn by Sertoli (1865), who called them "cellule ramificati." Most writers since his time have given them his name; but the term "follicle cell" (coined by Valette St. George) is frequently employed, as well as the term "foot cell" (J. E. S. Moore), while v. Ebner ('71) employed the name spermatoblast, and Benda ('94) that of vegetative cell. The name follicle cell is generally used for the cells composing the spermatocysts of invertebrates and lower vertebrates, and that of Sertoli cell for the physiologically correspondent cells of mammaliam testes.

As to the genetic relations of the Sertoli cells to the germ cells proper the writers fall into groups, which Waldeyer ('06) has designated as the dualists and the monists. The first of these regard the two kinds of cells as of entirely different origin, the spermatogonia proceeding from primordial germ cells and the cells of Sertoli from other elements. As dualists are to be classed Watase, Bardeleben, Benda, Waldeyer and Stephan. Watase ('92) and Bardeleben ('97) consider the Sertoli cells to be interstitial testis cells that have wandered into the seminiferous tubules; but Bardeleben's figures are quite indecisive, and Watase, in his very brief account of little over a page, drew his conclusions from the similarity in color of the cells of Sertoli and the interstitial cells after staining with cyanine, chromotrop and erythrosine. Though Bardeleben thus holds the two kinds of cells to be of different origin, he nevertheless thinks that the Sertoli cells give rise to "a rudimentary second form of spermatozomes." Benda ('94, '98), and Waldeyer relying upon him, considers the cell of Sertoli to arise from the indifferent cylindrical peritoneal cells. The dualists generally hold that a differentiated Sertoli cell remains functionally active during the life of the individual and does not regenerate more than the distal portion of its cell body; and they are also of the opinion that the cells of Sertoli proliferate themselves by division—amitotically, according to Bardeleben and Stephan, or mitotically according to Benda. On the other hand Prenant ('87), Schoenfeld ('01), Regaud ('99) and Bugnion ('06) consider both cells of Sertoli and spermatogonia to be derived from one kind of cells, by a process of division of labor; and this is in agreement with the results of most writers who have studied the origin of the follicular cells of the ovaries and testes of invertebrates—the follicular or nurse cell being generally regarded as a modified germ cell. Yet Regaud and Stephan ('02) hold that the fully formed Sertoli cells proliferate germ cells as well as nourish them; while Bugnion believes "the primordial spermatogonium gives place to a plurinucleate plate (part of the parietal synctium) which contains in a common cytoplasm spermatic nuclei and sertolian nuclei," after which the germ cells delimit themselves from the syncytium that remains as a Sertoli cell.

My conclusions differ practically in their entirety from those of the writers mentioned. In the human testis the cells of Sertoli are of common origin with the germ cells, one out of every four ultimate spermatogonia becoming a Sertoli cell. Sertoli cells are thus not differentiated from the germ cells merely in early fœtal history, but so long as ultimate spermatogonia continue to be produced. A Sertoli cell of man once differentiated does not, so far as I have observed, divide again, and consequently does not give rise to germ cells; further, a Sertoli cell dies completely after the spermatozoa that are associated with it depart from its surface, and it does not persist to nourish a second generation of spermatozoa. There being one Sertoli cell to every three definitive ultimate spermatogonia there is necessarily one to every twenty-four spermatozoa; accordingly, in man the number of spermatozoa, spermatic bundle, associated with one Sertoli cell cannot be "8 or 16" as Bugnion states.

But the point of the greatest interest with regard to the differentiation of the human Sertoli cell, is that it is determined by the inclusion of a peculiar cytoplasmic rod, this rod first arising in the antepenultimate spermatogonia. No such "Sertoli cell determinant" has been made known in any other object. In the case of the differentiation of the oögonia from the nurse cells in the ovary of the beetle *Dytiscus*, so well described by Giardina ('01), and corroborated by Debaisieux ('09), there is a remarkable mechanism of differentiation of the nurse cells: here the cells that are to become oöcytes receive a cast-off reticular part of the nucleus, while the cells which lack this extruded mass become nurse cells. It will be seen that this is an entirely-different process from that described by me for man, for in man the Sertoli cells are those that contain the differentiating body.

The development of the human Sertoli cell is clearly a very beautiful case of somatic differentiation. In fact, one may regard the multicellular organism as having two periods of somatic differentiation: the first when the tissue cells become differentiated from the germ cells, and the second, when in the early mass of germ cells, the primordial gonad, the Sertoli cells become differentiated from the germ cells proper. For the Sertoli cells may properly be classed as the soma of the testis.

Nothing like the rod that differentiates the human Sertoli cells from the other ultimate spermatogonia seems to be known in any other case of somatic differentiation. In the classical case of *Ascaris*, discovered by Boveri, prospective body cells cast off into the cytoplasm the ends of their chromosomes. In copepods and insects, according to Häcker and Silvestri respectively, a nucleolus or a mass of nucleolar substance thrown out from the germinal vesicle of the egg comes ultimately to lie in cells of the germinal cycle. The origin of the rod of the human Sertoli cells I could not determine, beyond that it is first apparent in the cytoplasm of the antepenultimate spermatogonia, and that it probably forms there during the rest stage of the cells. It comes to develop in all antepenultimate spermatogonia, therefore, before the distinction of Sertoli cells and germ cells; it becomes transmitted without division to one quarter of the ultimate spermatogonia, and that quarter transforms into Sertoli cells. Under these conditions, on account of the precision of the process, this rod must be regarded as a Sertoli determinant, and as a cytoplasmic and not a nuclear determinant. Whether the rod, or its substance, emanated in the first place from the nucleus, can be determined only by some fortunate observer who has more and better fixed material than was in my hands. But there is no reason to regard it as mitochondrial, as a chondriosome, because granular mitochondria have been described in mammalian Sertoli cells by Benda and others; in my material no mitochondria were seen in the spermatocytes and spermatids, they were evidently dissolved by the action of the fluid of Zenker, and it is therefore probable they were dissolved also out of the spermatogonia.

The rod that comes to determine the Sertoli cells increases in size while in the cytoplasm, becoming most voluminous in the ultimate spermatogonia; outside of the nucleus, also, occurs its process of abstriction of the primary rodlet and the division of the latter into the secondary rodlets. It is therefore clearly an extranuclear determinant of the Sertoli cell; and this as yet unique process of somatic differentiation seems to be controlled by an extranuclear body.

It has not been my intention to decide upon the function of the Sertoli cells. They increase greatly in size to produce a syncytial mass loaded with intracellular droplets, probably of fatty nature; they envelope closely the rapidly growing spermatocytes and for this reason they are generally supposed, and probably correctly so, to nourish this generation of germ cells. The fluid within the seminiferous tubules contains, so far as I have observed, neither erythrocytes nor leucocytes, therefore is probably derived from the droplets of the Sertoli cells and not from the blood serum. The spermatids at the commencement of their histogenesis lose their first connection with the Sertoli cells, while the nearly mature spermatozoa exhibit their heads buried in the substance of the Sertoli cells; the latter is then a second orientation of the germ cells to the Sertoli cells, one that cannot subserve nutrition, for the developing spermatozoa do not increase in size, but which is rather, as Loisel ('07) has shown, the expression of some chemico-tactile response. It may then be the Sertoli cells fulfill three functions: to nourish the spermatocytes, to furnish the fluid within the seminiferous tubules, and to attract the spermatozoa into oriented bundles.

It is certain that much more study is needed of the Sertoli cells, both from the standpoint of somatic differentiation as well as that of the physiology of the germ cells themselves.

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EXPLANATION OF PLATES I–V

All figures have been drawn by the author with the camera lucida at the level of the base of the microscope, and reduced one third in size in reproduction. Fig. 50 was drawn with Zeiss obj. C, ocular 12, all the others with the apochromatic immersion objective 1.5 mm., ocular 12.

The following abbreviations have been employed:

Id., idiozome.

R., rod.

r. 1, primary rodlet.

r. 2, secondary rodlets.

S.C., Sertoli cells.

Sp.G., ultimate spermatogonia.

Plate I.

Figs. 1–3. Entire antepenultimate spermatogonia.

Figs. 4–8. Rods of antepenultimate spermatogonia.

Fig. 9. A binucleate penultimate spermatogonium.

Figs. 10, 11. Penultimate spermatogonia.

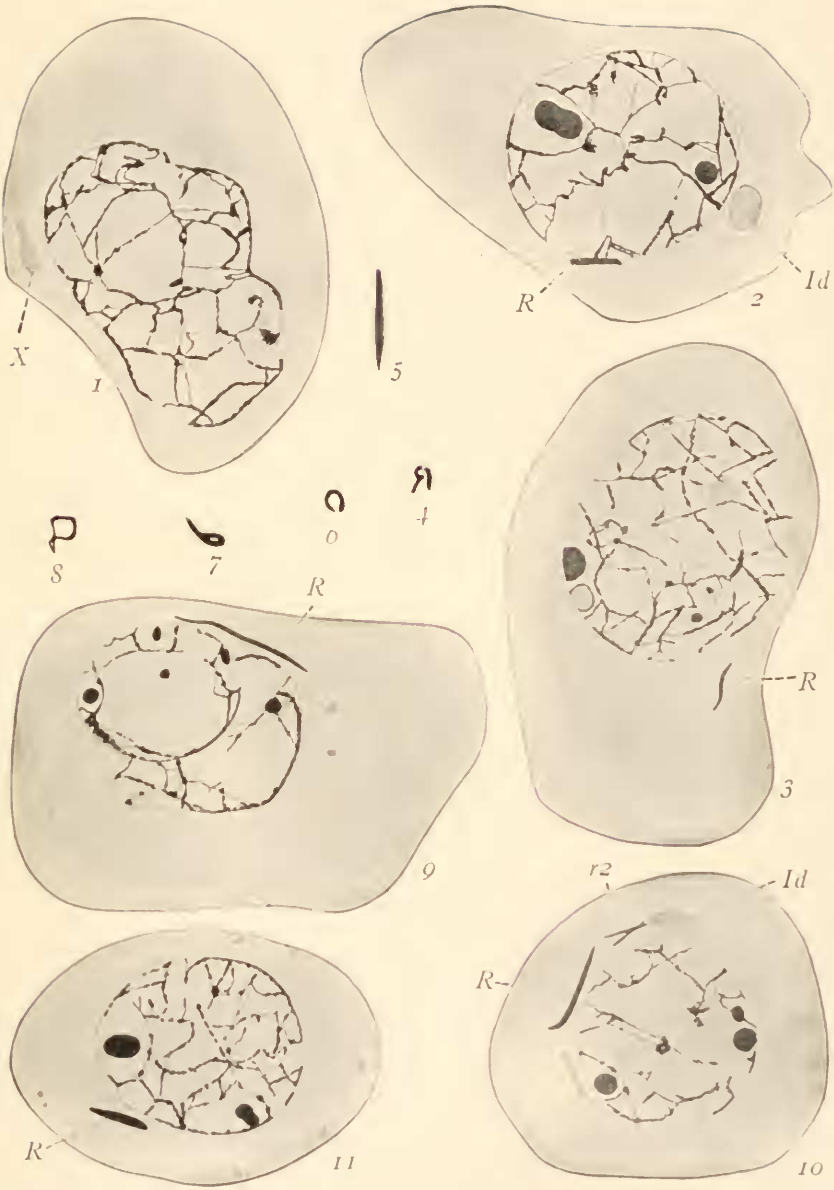

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Plate II.

Figs. 12, 13. Penultimate spermatogonia in division.

Figs. 14–16. Rods of penultimate spermatogonia.

Fig. 17. Quadrinucleate ultimate spermatogonium.

Fig. 18. Binucleate ultimate spermatogonium.

Figs. 19, 20. Ultimate spermatogonia.

Figs. 21–24. Rods of ultimate spermatogonia.

Fig. 25. An incipient Sertoli cell (*A*) next to two ultimate spermatogonia (*B*, *C*), and to a degenerate Sertoli cell (*D*). The inner margin of the wall of the seminiferous tubule is at the left.

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PLATE III.

FIGS. 26–30. Primary rodlet abstricting from the rod, early Sertoli cells.
FIG. 31. Trinucleate ultimate spermatogonium.
FIGS 32, 33. Early Sertoli cells with primary rodlets.
FIGS. 34–36. Secondary stages of Sertoli cells.

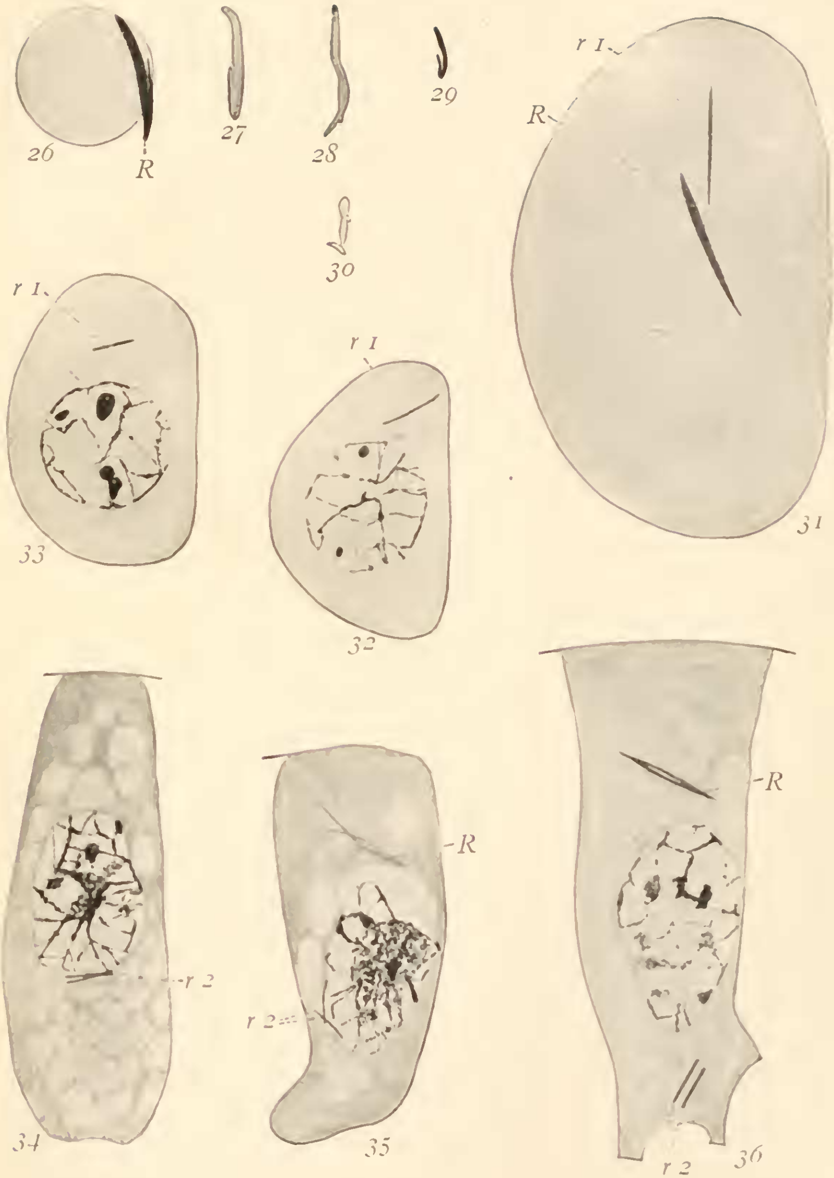

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PLATE IV.

Succeeding stages of Sertoli cells arranged in the order of the nuclear changes Fig. 40 exhibits a dividing rod of a stage similar to tha of 41.

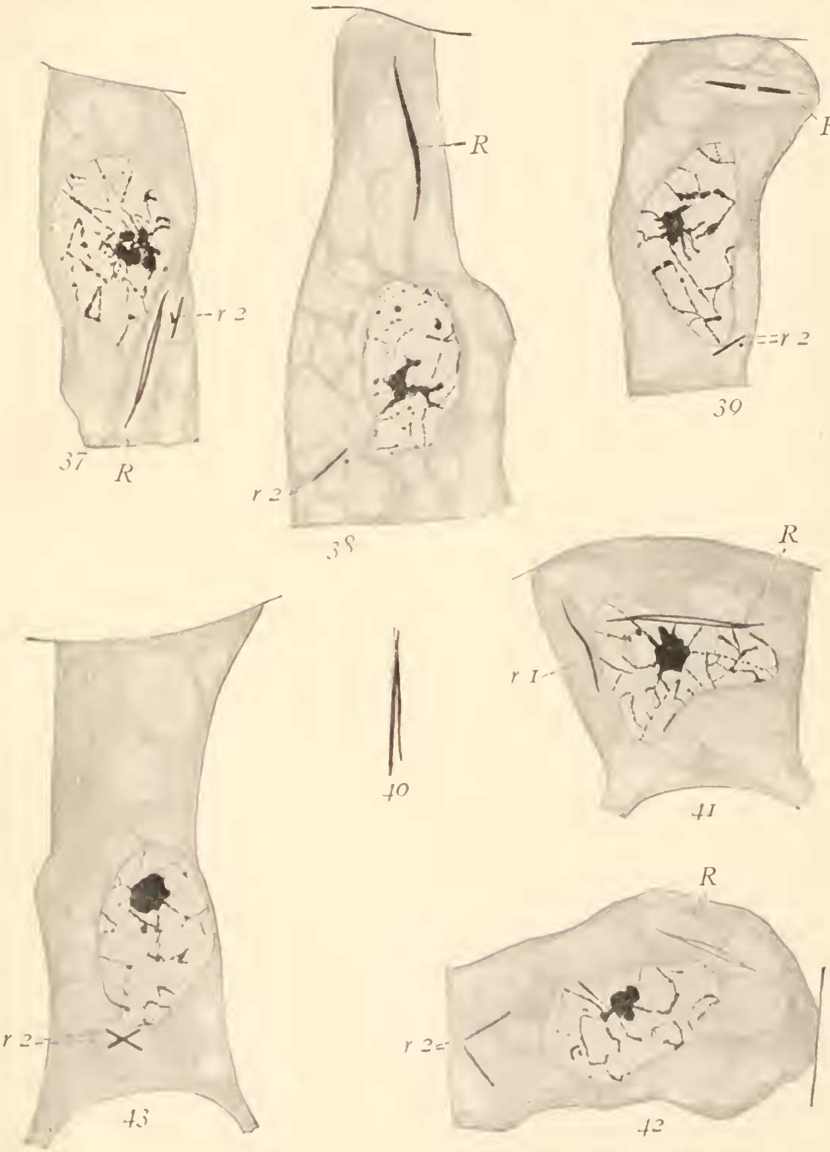

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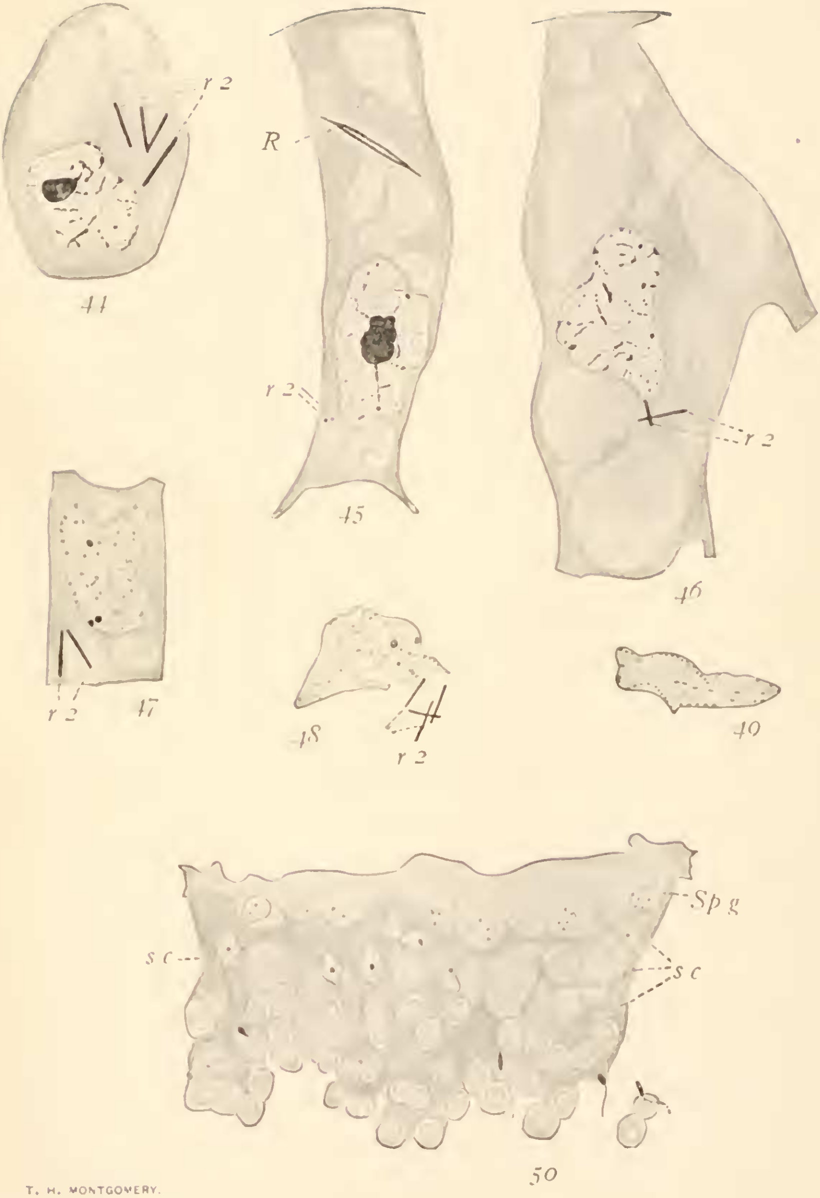
PLATE V.

FIGS. 44–47. Later stages of Sertoli cells, Figs, 44 and 47 being oblique trans-sections.

FIGS. 48, 49. Degenerate nuclei of late Sertoli cells.

FIG. 50. Portion of a section of a seminiferous tubule. Uppermost is the wall of the tubule, and next to it a layer of ultimate spermatogonia. The syncytium of the Sertoli cells is expressed by dark shading, and their nuclei are distinguishable by their angular form and central chromatic body. The other cells shown are chiefly early spermatids.



T. H. MONTGOMERY.

ON THE CAUSE OF AUTOTOMY IN TUBULARIA.

OSCAR RIDDLE.

In the course of studies on the oxidizing and reducing powers of the various tissues and body regions of hydromedusæ the phenomenon of autotomy was observed to occur so generally and so rapidly in *Tubularia* as to invite attention to its cause. That *Tubularia* is capable of autotomy—*i. e.*, of the self-division of its body—has long been known, the process having been observed by Giard, Loeb, Driesch, Morgan, and others. Two investigators have definitely sought to determine the *cause* of the phenomenon. The conclusions of these two workers seem, however, not to be in accord. Godlewski[1] maintains that degeneration of the hydranth precedes and conditions the autotomy, so that it is a *degenerated* hydranth that is severed from the body. Morse[2]—who seems unfortunately to have overlooked Godlewski's paper—writing in this journal states that autotomy may occur without an initial degeneration in the hydranth, if one can judge of this from histological examination.

My own experience with autotomizing *Tubularia* indicates that when this process is effected very slowly and gradually, as is ordinarily the case, one can certainly sometimes find, as did Godlewski, that before the actual separation of the hydranth the latter has undergone considerable degeneration. On the other hand, I have had many autotomized hydranths to live in apparently perfect condition for three and four days. Godlewski himself notes one such hydranth which lived for two days. It is quite easy, too, to confirm Morse's statement that a rise in temperature favors the occurrence of autotomy. Nevertheless, the peculiar conditions under which I was able to observe the autotomy convinced me that neither of the above mentioned supposed causes, nor yet both combined, is the immediate or

[1] Godlewski, E., "Zur Kenntnis der Regulationsvorgange bei *Tubularia mesembryanthemum*." *Roux's Archiv*, Vol. 18, 1904.

[2] Morse, Max, "The Autotomy of the Hydranth of *Tubularia*." BIOLOGICAL BULLETIN, Vol. 16, 1909.

adequate cause of autotomy. I therefore gave a little special attention to this subject, the results of which may be summarized here.

The particular experience which seemed to contravene the proposed causes as being the actual immediate cause was the following: If normal healthy individuals of *T. mesembryanthemum* be held by the lower stem or stolon and drawn through any one of a variety of solutions—sodium tellurite, sodium selenite, etc.—*complete autotomy may occur in less than one minute!* Clearly degeneration is not the cause of the autotomy in these cases. Other members of the colony taken from their moorings and placed in vessels supplied with fresh sea water remained for days without autotomy. When some of these were similarly drawn through pure sea water they remained intact without autotomy. It was found, moreover, that the autotomy likewise occurred even when the animal was dipped into a solution of Na_2TeO_3, $NaSeO_3$, etc., of *lower* temperature than that from which it had just been removed. Here, too, the autotomy was rapidly and decisively effected. In these cases the autotomy plainly could not have been caused by a rise in temperature; the temperature change actually being in the opposite direction. In many cases the animals were removed from water at 16° C. and drawn through a solution at 13° C.

In order to study the changes occurring in the rapidly autotomizing animals these were examined with a Zeiss binocular while being drawn through the solutions. By this means it was found: (1) that as soon as the animal touches the solution there follows a very *strong contraction* of tentacles, hypostome, peristome, etc.; (2) that the "neck" region becomes *extremely contracted* and narrow, and apparently so much weakened as to be unable longer to support the weight of the hydranth; or rather too weak to sustain the slight pull on the hydranth as it is being drawn through or lifted from the solution. The appearance here is such as to indicate that the contraction of the circular fibers of this region is of sufficient force, not only to close completely the central channel, but also to separate and crowd out many entoderm cells, and likewise to weaken their own adhesion and that of the other ectoderm cells to each other. *A vigorous con-*

traction in response to stimulation therefore seems to be the effective cause of autotomy.

It has been possible in a few instances to get a beautiful demonstration of the strength of the contraction in the "neck" region. If a tubularian be found with the gastro-vascular cavity of the hydranth well expanded and full, it can sometimes be induced—by pricking the peristome—to contract the peristome first, and thus retain the whole of the fluid of the cavity. In this condition the hydranth somewhat resembles a rubber ball; the channel to the outside being closed by the contracted peristome, and the posterior continuation with the cavity of the stem or body being interrupted by the above-mentioned contraction of the "neck" region. If now, with an appropriate blunt instrument, pressure be brought to bear upon this sphere, and the whole proceeding carried out under the binocular, one can watch everything and gauge with one's own muscles the strength of the contraction in the peristome and "neck." In the instances where I have carried out this experiment *I have never been able to force the opening of the channel in the neck region.* Some part of the hypostome wall is the first to break.

It is, too, this very strong contraction that carries the cœnosarc of the *neck region* quite away from the perisarc (see Fig. 0). Probably the reason that the point of the autotomy is always so definitely localized in this "neck" region—as was first recognized by Giard[1]—is that the remainder of the slender portion of the animal is covered with a chitinous perisarc which is rather impermeable and highly protective against stimuli.

In "normal" cases—those in which the process of autotomy is extended over a period of several hours or a day or two—it is well known that a very complete histolysis of the cells in the "neck" region occurs. There is good evidence however that in these cases, too, the histolysis is preceded by a rather strong or by a prolonged contraction of this region. In very weak solutions of tellurium and selenium salts, of acids and alkalis, and even by watchful mechanical stimulation of the animal into continuously contracted state, I have been able to effect the

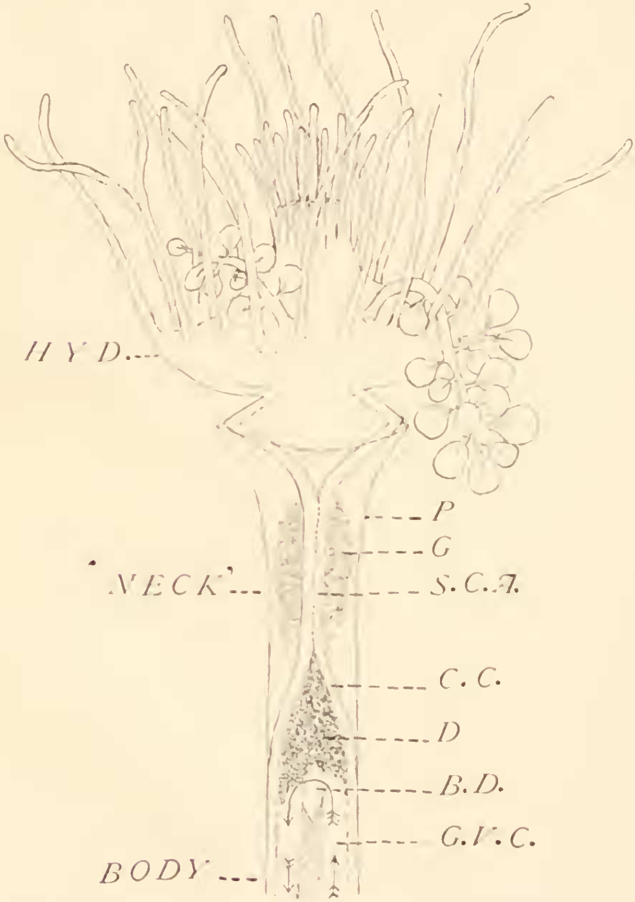
[1] Giard, A., "L'Autotomie dans la série animale." *Revue scientifique*, p. 629, 1887.

autotomy of healthy hydranths more gradually in periods extended to one to four hours, and to watch the course of the process in a single individual during this time. From these observations I may here record one or two points which seem to throw some light on the reason for the histolysis just mentioned.

I cite the case of a tubularian which was kept mechanically stimulated by touching or pricking the hydranth with a dissecting needle and in which the process of autotomy had advanced at such a rate as readily to separate at the end of four hours when lifted from the sea-water. With the beginning of contraction in this animal the circulatory current in the "neck" region was stopped; indeed the circulatory-nutritive fluids were quite expelled and excluded from approximately two millimeters of this region; the entodermic walls of the tube here being completely and tightly apposed. The closure at this point also largely stopped for a time the circulation through the stem. The fluids, however, were as before in *contact* with the walls of the gastro-vascular cavity everywhere except in the much contracted neck region. That is to say, in the contracted animal the normal nutritive fluids were in contact with all the structures with which they are normally in contact except at one point—the "neck" region; *it is always at this latter point that histolysis and autotomy later occur.*

In a little less than an hour it was found that the dissepiment which divides the gastro-vascular cavity of the stem into two channels—one for the anterior, the other for the posterior flow of the circulatory fluids—had been broken at a point a little below the contracted "neck," and that the usual *circulation* of fluids was again established within the stem. Soon however there accumulated at the point immediately below the neck a quantity of the red pigment and other débris from the circulation; thus the channel became so firmly plugged that even a relaxation of the contracted neck region could not now effect a reëstablishment of circulation in this "neck" region.

It is my opinion, furthermore, that the reëstablishment of this circulation after a few hours of contraction might be prevented or at least greatly hindered by another circumstance if for any cause the débris just mentioned should fail to collect and act as an effective block. I refer to the accumulation of a gelatinous mass between the perisarc and cœnosarc which begins to be secreted by the cœnosarc soon after it pulls away from its contact with the perisarc. The secretion is perhaps of the nature



Representing the condition leading to autotomy in a tubularian. Such conditions are present in *Tubularia* which have been kept stimulated from one to several hours. *Hyd* = hydranth; *B.D.* = break in dissepiment; *C.C.* = cœnosarc or body wall; *D.* = debris left by circulating current; *G.* = gelatinous mass secreted by contracted cœnosarc; *G.V.C.* = gastrovascular cavity of body; *P.* = perisarc; *S.C.A.* = strongly contracted area = "neck."

of material for a new perisarc, and as noted by Godlewski it hardens on contact with water. The accumulation and hardening of this mass would probably make a reopening of the closed channel very difficult or quite impossible.

We see then that the contraction of the contractile parts of *Tubularia* acts differentially upon its various organs. Such contraction does not rob the hydranth of its contained fluids, nor of its ability to circulate these fluids. The same is true for the stem region, except that there the actual movement of the fluids is in abeyance for a very short time. The contraction which occurs in the neck region, however, brings about far different relations between the contracting area and the nutritive medium. Here there results, not only a cessation of the circulation of fluids, but a complete loss of contact with these fluids following the complete closure of the channel; whilst finally the breaking of the dissepiment immediately below the neck region, and the subsequent plugging of the end of the connecting channel with pigment and débris, preclude the possibility that such contracted region may again regain its circulation together with the food it brings. This area then necessarily disintegrates; and the break—the autotomy—necessarily occurs at this the weakest point.

Our conclusion is that autotomy in *Tubularia* is the result of the contraction of the animal; similar but weaker contractions being common and central features in the behavior of the animal. If the contraction be either too strong, or too much prolonged, autotomy will follow. That is to say, if a very slight strain be put upon the "neck" region while its circular fibers are in a state of extreme contraction separation results at once. If the contraction be not so strong, but considerably prolonged, readjustments are effected in the circulation which prevent the ingress of food to the contracted "neck" region. Degeneration now occurs in this region and the break—the autotomy—follows at this same point. There is, then, no great mystery attached to "l'amputation spontanée"; not even a complex organic correlation to direct a watchful and sacrificial neck in severing an offending head from an unoffending body.

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